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PRESS

THE DEVELOPMENT OF THE PECTORAL LIMB OF NECTURUS MACULOSUS

WITH ELEVEN PLATES

BY

HSIN KUO CHEN

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I. INTRODUCTION

Previous work on the development of the chiropterygious type of limb in vertebrates has been carried on in connection with various problems, which may be divided for convenience into three classes:

(1) The earliest papers dealt chiefly with the origin of the primordial limb bud and its relation to the myotomes. The results obtained, however, vary with the different investigators, and on the basis of the results obtained, the workers may be divided into two groups. The first group, including Goette ('75), Jordon, ('88), Kaestner ('93), Field ('94), Mollier ('95), and Tschernoff ('07) thought that the myotomes take part in the formation of the limb bud either in the form of processes as the muscle buds in fish, or by way of cell migration as in the case of Bombinator, described by Goette. The second group of investigators, including Paterson ('88), Harrison ('95, '18), Byrnes ('98), Lewis ('10), and Detwiler ('18), claimed that both observations and experimental methods show that the limb arises as a thickening of somatopleure and is in no way derived from the myotomes or their processes. None of these investigators have attempted to trace the limb development as far as the stage where it is comparable with adult conditions.

(2) Considerable work has been done recently by experimental methods in analyzing the factors which determine the differentiation of the limb bud, the change of symmetry, and the formation of the nerve plexus in transplanted limbs. Investigation in this field has been carried on chiefly by Harrison and his students (Harrison '07, '15, '16, '18, '21, '25; Detwiler '18, '19, '20, '22; and Swett '23, '26).

(3) A new theory concerning the development of the limb musculature in tetrapods has been advanced recently by Romer ('27). According to his theory the muscles of the chiropterygious type of limb may be arranged into two groups, one dorsal (dorso-medial) and one ventral (ventro-lateral). These groups of muscles may be homologous with, or derived from, the two opposed masses of muscles present in the paired fins of fish. This theory was at first based upon his comparative studies of several primitive adult forms of tetrapods ('22, '24), and was later supported by his embryological work on the development of the thigh musculature of the chick ('27).

It was suggested to the writer by Professor Waldo Shumway that a study of the development of the limb in one of the lowest tetrapods, from its earliest appearance to the stage at which it attains the adult form, might give us a better understanding of the relations of the myotomes to the early formation of the limb bud, and the homology of the appendicular musculature with the two opposed masses of muscles in the fish

fin from the embryological point of view. Necturus was chosen as the subject for this investigation because of the accessibility of the material. The results obtained in this investigation are presented in the following order: (1) the formation of the primordial limb bud and its relations to the myotomes; (2) the differentiation of the limb bud, in which the primordia of the skeleton, musculature, and nerve plexus are laid down; and (3) the further development of the primordia which ultimately attain the conditions similar to that of the adult.

This work was undertaken under the supervision of Professor Waldo Shumway, to whom I am indebted for advice and criticism. I also wish to express my gratitude to Dr. H. W. Hann for helpful suggestions; to Dr. A. R. Cahn for his kindness in placing a large collection of preserved specimens at my disposal; and to my wife for her ever-ready help during the completion of this investigation.

II. MATERIAL AND METHODS

The embryos and larvae of Necturus maculosus were collected at Oconomowoc Lake, Wisconsin, in the summer of 1925. They were killed and fixed in Smith's fluid which was prepared according to the following formula: potassium bichromate .5 gram, glacial acetic acid 2.5 cc., formalin 10 cc., and distilled water 75 cc. The specimens were later preserved in 70% alcohol until ready for use. Serial sections 20 μ in thickness were cut in three different planes by means of the paraffin method. The animals that were sectioned ranged from 9 mm. in length where the primordium of the limb bud is just discernible, to 34 mm. where the structures of the limb are comparable with the adult stage. The sections were stained in a saturated aqueous solution of basic fuchsin for five minutes, and after being rinsed in water, were transferred to equal parts of a saturated solution of indigo-carmine and a saturated solution of picric acid for twenty minutes or more until the sections appeared green (Shumway '26). From this solution the slide was passed rapidly from 70% alcohol to the pure xylol where a bright green indicated that the tissues were well differentiated. Otherwise the sections were decolorized in acid alcohol and restained. In a well-stained section, the following color differentiation appears: precartilage, bright blue; cartilage, deep blue; muscle, grass green; nerve, dark purple to light gray; connective tissue, bluish gray; blood corpuscles and mitotic figures, deep red.

The following table shows the specimens that were sectioned by the writer with the indication of their approximate age and number of somites according to the tables of Eycleshymer and Wilson ('10).

Length in mm.	*Age*	*Somites*
9 Trans.	23 days 10 hrs.	20-22 pairs
10 Trans.	24 days 22 hrs.	23-24 pairs
12 Trans.	28 days 2 hrs.	28-29 pairs
13 Trans. (3 series)	30 days 8 hrs.	30-31 pairs
Sagit.		
Front.		
14 Trans.	32 days 10 hrs.	31-32 pairs
Sagit.		
Front.		
15 Trans.	34 days 12 hrs.	32-34 pairs
Sagit.		
Front.		
16 Trans.	36 days 16 hrs.	36-38 pairs
Sagit.		
Front.		
17.5 Trans.		
18 Trans.	40 days 2 hrs.	44-46 pairs
Sagit.		
Front.		
19 Trans.	43 days	48-50 pairs
Sagit.		
Front. (2 series)		
20 Front.	46 days 2 hrs.	50-55 pairs
21 Trans.	49 days	
Sagit.		
Front. (2 series)		
23 Trans.	55 days	
Sagit.		
Front.		
27 Front. (2 series)	67 days	
32 Trans.	87 days	
Front.		
34 Trans.	97 days	
Front.		

In addition to the above sections the writer has had access to the large collection of slides of Necturus maculosus used by Eycleshymer and Wilson in preparing their important monograph on "Normal plates of the development of Necturus maculosus" ('10). From all these slides a careful study of embryos and larvae of different stages was made. All drawings of structures at their important developmental periods were outlined by the aid of the camera lucida. Besides the sections, Born's method of wax reconstruction was also employed to study the developing limb as a whole. This facilitates the identification of the tissues separated by different sections, and furnishes a clear picture concerning the degree and extent of the development of various structures in relation to one another. Models prepared by DeBruine ('28) were compared with those made especially for this investigation.

The terminology applied to the skeleton and musculature of the

limb in Amphibia varies with the different authors. In order to avoid confusion the nomenclature employed by Wilder ('03, '12) is adopted in the following descriptions.

III. THE PRIMORDIAL LIMB BUD

1. Structure of the Undifferentiated Mesenchyme

The earliest stage at which the mesenchyme cells appear in the limb is in the embryo 9 mm. in length (Fig. 1). They are found on the ventro-lateral side of the myotome and scattered among the numerous yolk granules, which vary in size but are quite uniformly distributed throughout the whole region. The form of the cell (Fig. 7) varies somewhat, the round and oval being the most common, with oblong and polyhedral ones occurring occasionally. The main bulk of the cell is composed of nucleus. The membrane of the nucleus is well defined, and terminating in it are the delicate chromatin threads. The threads form a fairly regular meshed reticulum. Imbedded in it are several nucleoli and many small chromatin bodies. At this stage, the mitotic figures are rarely observed. The whole bud appears as a slightly elevated ridge at the surface of the body wall. In embryos between 10 and 11 mm. in length (Figs. 2, 3), the mesenchyme cells increase slightly in size and number. Their chromatin network is more clearly shown and associated with it are several deeply stained karyosomes. The majority of the cells are now oval in shape and only a small number of them are round or polyhedral. The yolk granules, as before, are different in form —round, oval, and quadrate—and are still thickly and evenly distributed in the limb region. The general outline of the limb is not much different from that of the preceding stage.

As the development proceeds, the limb bud is more prominently marked. At the 12 mm. stage (Fig. 4), the mesenchyme cells form a rather compact mass and push out from the surface of the body wall. At this time, the decrease in the number of yolk granules commences, and the mitotic figures are frequently seen, indicating the beginning of active growth. The structure of the nuclei, however, does not widely differ from that just described.

In the succeeding stage, great changes occur in the limb region. In an embryo 13 mm. in length the yolk granules have almost entirely disappeared and the mesenchyme cells tend to collect together. A great many of the nuclei are still oval, but an increase in the number of cells and the numerous mitotic divisions observed at this stage mark the period of active growth.

This period of active growth extends to the next stage when the embryo is 14 mm. in length (Figs. 8, 9), when a noticeable change occurs involving the elongation of many nuclei into cylindrical form. Along with this change, the chromatin network is very clearly revealed, and its meshed reticulum becomes larger with a considerable increase in the number of threads. Among the meshes there are several nucleoli seen as small deeply staining bodies.

Following this stage, the mesenchyme cells in the limb bud, on account of the active multiplication, show a slight tendency to divide into peripheral and central groups (Fig. 10). This phenomenon is marked by the growth of the blood vessels in the region between these groups. In a transverse section through the limb area of a 15 mm. embryo, the spaces between these groups occupied by blood vessels are quite noticeable. The cells on the peripheral or the outer zone as seen in transverse sections are usually cylindrical with their longest axes radiating from the center of the limb, while those in the central or inner region are commonly oval in shape. Externally these mesenchyme cells are inclosed by the epidermis, which is two layers of cells in thickness.

Through this period of growth, the mesenchyme cells in the central area begin to differentiate into cartilage, and those belonging to the peripheral part later give rise to the muscles. The details of these processes will be taken up in their respective sections.

2. Relation of the Myotomes to the Anterior Limb Bud

The formation of myotomes takes place much earlier than that of the anterior limb bud. In embryos with about twenty pairs of myotomes (9 mm. Fig. 1), the primordium of the limb bud becomes discernible as a thickening of the somatopleure below the myotomes. It consists of an aggregation of a few mesenchyme cells embedded among the numerous yolk granules and covered externally by a layer of epidermis. The ventral border of the myotomes in the limb region as seen in transverse sections is somewhat rounded, extending to the level of the center of the notochord without showing any indication of the presence of their ventral process. The cells in the myotomes are commonly round or oval, and packed together with yolk granules. At this stage, the process of fibrillation has already begun, and the delicate threads are easily found, especially at the ventral side of the myotome. Soon after the appearance of the anterior limb bud, the rudiment of the pronephros may be seen in the region medial to the dorsal part of the limb bud. The pronephric tubules are composed of a few cells densely laden with yolk

granules, and show a somewhat tubular arrangement. Thus at the outset, the limb bud and the pronephros are closely associated.

In a slightly later stage, the myotomes in the anterior trunk region grow downward. In the embryo with about 24 pairs of myotomes (10 mm.), the myotome processes begin to appear on the ventral outer-border and soon extend to the level of the lower margin of the dorsal aorta (Fig. 2). The cells in the ventral processes become elongated in the direction of their growth. Simultaneously with the development of the ventral processes, the pronephroi become more definite in form, with their cells arranged into tubular outline inclosing a cavity in the center. The yolk granules previously scattered around are now confined in the walls of the tubules. In a transverse section of a 10 mm. embryo through the limb region, the pronephric tubules are shown on the dorso-lateral border of the somatopleure a little below the distal end of the myotome process. Hence in the next stage, when the ventral processes elongate farther downward, they meet the pronephric tubules and grow along their lateral side, medial to the limb bud. This is shown in a transverse section of an 11 mm. embryo (Fig. 3), in which the tips of the myotome processes have already grown over the pronephric tubules and entered the dorsal region of the anterior limb bud. This indicates that the close proximity of the myotome processes and the limb bud is brought about by the outgrowth of the pronephric tubules, which at this stage elongate at right angles to the direction of the growth of the ventral processes. In spite of their close association with the mesenchyme cells of the limb bud, the cells of the myotome processes can be easily distinguished by their arrangement in double strands with the longest axes of their nuclei lying dorso-ventrally in the direction of their growth, while the cells in the limb region do not follow any definite arrangement. This characteristic difference in the cells of the myotome processes and of the limb bud make it easier to determine whether or not there is any migration of cells from the former to the latter. Close examinations have not disclosed any mingling of cells from these two separate sources, nor is there any indication of migration of cells from the myotome derivatives to the limb bud. This strongly suggests that at least in the group of urodeles the evidence supporting the assumption that the mesenchyme in the primordial limb is derived from the myotomes is far from being conclusive.

The character of the independence of the limb bud from the myotome derivatives is more clearly shown in the succeeding stage when the myotome processes have reached into the ventral base of the limb bud, and appear in transverse section as a long band consisting of two or three columns of nuclei closely packed together. Their well-defined gen-

eral outlines, together with the dorso-ventrally elongated nuclei and deep-staining capacity, make them readily recognizable from the surrounding structures. In a transverse section of a 12 mm. embryo (Fig. 4), it is seen that between the medial margin of the limb and the ventral myotome process there exists a clear space in which no trace of cells is found. Such clear space naturally indicates the separation and independence of the limb bud from the myotome derivatives. The assumption that there is a migration of cells from such a well-isolated myotome process to the limb region without any trace of cells connecting them is hardly probable. On the other hand, it does show that the cells in the limb bud multiply and differentiate in situ, as manifested by the frequent occurrence of mitotic figures among them, and by their increase in number and size at the time when the influence of the myotome processes does not reach to the limb region. This situation becomes more conspicuous as development progresses.

In embryos between 13 and 14 mm. in length (Fig. 9), the pronephric tubules elongate greatly in the direction of the limb bud and become convoluted. By their excessive growth, they force their way through the ventral myotome processes and come to lie near the base of the limb. In this manner, the ventral processes are cut off from their respective myotomes. Posterior to the pronephric region, the connection between the myotomes and their processes still persists. In the meantime, the cells in the limb region enter the period of active proliferation and numerous mitotic figures may be seen. As a result the limb bud grows out on the surface of the body wall. On the other hand, the isolated ventral myotome processes show little sign of cell division, and mitotic figures are only occasionally observed. The characteristic dorso-ventral elongation of the cell persists a while, but is soon lost. The myotome processes appear now as a thick band of cells bordering on the outer margin of the coelom at the ventral base of the limb. A comparison of the numerous cases of cell division in the limb region, with that of a few instances occurring in the ventral processes, can hardly convince one that the source of the limb material depends upon the myotome derivatives. Instead, it indicates that the limb-forming substance is differentiated in situ rather than it is derived from the myotome processes.

In a transverse section of a 14 mm. embryo (Fig. 9), it is shown that the cells in the limb bud are crowded together at the central part, but become thinner toward the base or proximal region. The cell divisions are very active in the area where the cells are most crowded. The isolated ventral process, as shown in the section, extends from the middle part of the medial margin of the limb, to its ventral base. Its general form is so well defined and distinguished that it does not show any feature of

intermingling with the nearby mesenchyme cells. The cells in the process are either oval or elongated and mixed together with yolk granules. They are confined within the boundary of the process. The region of the limb close to the ventral process is filled with only a few loosely distributed mesenchyme cells. Mitotic divisions occur less frequently here than farther away. Comparing the conditions found in the myotome processes and in the region close to them with those seen more distant from them, one is justified in saying that the myotome processes do not take part in the formation of the limb. In the same section, the excessive growth of the pronephric tubules is clearly shown. They become convoluted and push out farther than the outer lateral margin of the myotome. The myotome processes after being constricted off by the pronephric tubules are pressed outward closely against the base of the limb.

In subsequent stages (Figs. 10, 11), differentiation of the limb bud and the final fate of the myotome derivatives conclusively show that they are independent of each other. At the time when the blood vessels appear in the limb region, the cells in the limb bud multiply rapidly and begin to separate into the peripheral and central groups. The peripheral cells later give rise to the muscles, while the central ones form the first center of chondrification. During this period of remarkable changes in the limb region, the isolated ventral myotome processes gradually increase in bulk and grow downward along the outer margin of the somatopleure. In an embryo 17 mm. in length when the humerus enters the period of chondrification, the process of fibrillation has taken place in the myotome processes which are soon transformed into the definite ventral muscles of the body wall along the outer border of the coelom.

The tracing of the ventral myotome processes from their earliest appearance to the stage at which the definitive structures are derived from them warrants the foregoing statement that the limb-forming mesenchyme is differentiated in situ and is in no way derived from the myotomes or their processes. The evidences supporting this statement may be summarized as follows:

(1) The mesenchyme cells of the primordial anterior limb bud are found as the thickening of the somatopleure before the appearance of the ventral myotome processes.

(2) During development, the pronephric tubules elongate at right angles to the direction of the growth of the ventral myotome processes and press the latter closely against the medial side of the limb bud. The proximity of the limb bud and the myotome processes is merely due to the excessive growth of the pronephric tubules.

(3) During their close contact with the limb bud, the myotome processes are distinguished from the surrounding mesenchyme cells by

their dorso-ventrally elongated nuclei, by their well-defined general form, and by the existence of a space between the ventral processes and the base of the limb bud. No indication of a migration of cells is found.

(4) When the ventral processes are cut off from their respective myotomes, they grow downward along the outer border of the coelom and later transform definitely into the ventral muscles of the body wall.

IV. DIFFERENTIATION OF THE LIMB BUD

1. Formation of Cartilage

A. Differentiation of Chondroblasts and Intercellular Matrix

It has been mentioned in the previous sections that in the early stage the limb bud is composed of a mass of undifferentiated mesenchyme cells extending from the third to the fifth myotomes (Figs. 5, 6). The first indication of the differentiation of chondroblasts takes place in a slightly later stage by a condensation of the cells in the center of the limb bud (Fig. 11). This condensed mass of cells forms the first center of chondrification and represents the blastema of the future humerus. The cells in the blastema possess at first the same form as the undifferentiated mesenchyme, but soon they become elongated. In a frontal section of a 16 mm. embryo through the limb region (Fig. 12), it is seen that the elongated cells at the center of the condensed mass are arranged in transverse rows at right angles to the long axis of the cell mass. At the periphery of the condensed mass, the embryonic chondroblasts merge imperceptibly into the surrounding undifferentiated tissue. From this center of condensation, growth of the limb skeleton proceeds both proximally and distally. Proximally, the cells in the girdle region are soon converted more or less simultaneously into the blastema of the future girdle. Distally, at a slightly later stage, centers of chondrification appear respectively in the ulna and radius. In embryos 18 mm. in length (Figs. 15, 16, 17), rudiments of all parts of the limb skeleton become distinguishable.

Following this incipient condensed stage, the next step in the development of cartilage involves the differentiation of the intercellular matrix. This process is first indicated by the appearance of narrow spaces among the condensed cells. At its beginning, this ground substance is stained light blue by picro-indigo-carmine and its structure is indistinguishable. It appears as a layer of homogeneous, transparent substance over the surface of the closely aggregated chondroblasts. The character of the matrix, however, becomes clearer and clearer in the later stages as it

increases in staining capacity and the chondroblasts move apart from one another. It is now stained deep blue and forms the distinct capsule surrounding each cell. As development progresses, the matrix increases in thickness through the constant secretion of the ground substance and is later clearly revealed as a close network of fine fibers. The assumption made by the early investigators that the matrix of the hyaline cartilage is made of a simple homogeneous material now becomes untenable.

B. Growth of Cartilage

During the period of rapid development, the cartilage increases in length and thickness. This is brought about by both interstitial and appositional growth. By interstitial growth, the cells within the cartilage increase by mitosis. In many bases, after division, two of them are found in the same capsule. When they move apart, a partition, very thin at first, appears between them. These daughter cells may soon divide again. As the cell divisions go on, the new ground substance is constantly laid down, and the cells tend to push themselves apart from one another. In addition to this process of development, the cartilage also increases in size by appositional growth through the formation of new cartilage over the external surface. As it has been mentioned in previous sections there is a zone of undifferentiated cells around the peripheral region of the cartilage. These cells are usually in the process of active cell division. Those nearest to the cartilage first undergo the process of chondrification by which they are transformed into chondroblasts and incorporated into the cartilage. This process constantly takes place during the period of rapid development, and the new cartilage is continuously differentiated and added to the surface of the old layer.

Along with its growth in length and thickness, the cartilage simultaneously differentiates into three zones of cells (Fig. 22). Their mode of development is closely similar to that observed by Fell ('25) in the case of the fowl. In Necturus, the earliest stage at which the differentiation of the three zones of cells takes place is in the embryo about 19 mm. in length. The characteristic formation of these zones is most easily seen in a frontal section of the limb. The first zone of cells occurs in the center, or diaphysis, of the cartilage. The cells are characterized by their polyhedral shape with large lacunae and thickened capsules. Some cells in this zone are shrunken, manifesting the tendency toward degeneration. The second zone consists of longitudinally flattened cells lying on both sides of the first zone showing the process of active division. The third zone is confined to the epiphysial region in which the cells are distinguished from the others by their round or polyhedral outline. They are smaller in size and closely arranged in an elliptical form. In this

region, mitosis is most active. Between the first zone or toward the end of diaphysis and the beginning of epiphysis are found several elongated cells arranged in a transverse line.

During the later stages of development several changes occur in these three zones of cells (Fig. 26). The changes are most pronounced in the older larvae. In zone one, especially at the center of the diaphysis, the matrix appears as a piece of spongy network with heavily thickened capsules. The cells embedded in the capsules become greatly shrunken —which results in the formation of lacunae. In zone two the formation of matrix begins among the flattened cells, where at first numerous slender strands are formed which finally become dense and possess the same appearance as those in zone one. In zone three, the epiphysial region is now seen as a close network with numerous cells distributed within the dense matrix. The cells in this region now change their elliptical arrangement and take on the concentric configuration. This is most noticeable in the head of the humerus which shows a sort of ball-like form fitting into the cavity of the girdle.

C. Regional Chondrification of the Limb Skeleton

Starting from the first center of chondrification, it has been found that the earliest blastema formed from the undifferentiated mesenchyme represents the rudiment of the future humerus. At first, it is simply a mass of condensed cells with uniform caliber throughout. As soon as it enters the period of chondrification, the differentiation of diaphysis and epiphysis commences (Fig. 15). The epiphysis, or the extremity of the humerus, is comparatively broad with its surface surrounded by undifferentiated cells, but is demarcated from them by a layer of flattened chondroblasts. When the embryo is about 20 mm. in length, the formation of three zones of cells as previously mentioned takes place.

During the rapid growth of this cartilage, rotation of the limb occurs. Before the chondrification of the humerus, the whole limb bud grows out from the body surface at right angles to the long axis of the embryo and is marked with two borders and two surfaces (Fig. 11). The anterior or the preaxial border directed toward the head of the embryo corresponds to the radial border of the adult tetrapod limb. The posterior or postaxial border directed toward the tail corresponds to the ulnar border of the adult. The upper surface facing dorsally in relation to the embryo is known as the extensor surface. The lower surface turned ventrally is known as the flexor surface. During the process of rotation, the radial or preaxial border becomes ventral, directed toward the body. The ulnar or the postaxial border becomes dorsal, directed toward the cephalic end. The whole limb inclines posteriorly with the flexor sur-

face of the hand pressed against the body. Along with the rotation of the limb, a pronounced bending of the humerus takes place. In a frontal section of the limb at this stage, it is seen that the convex aspect of bending is directed toward the ulnar border, and its concave aspect toward the radial side. Through the formation of this bending, a constriction appears between the diaphysis and epiphysis, making their division more clearly marked.

The next step of chondrification occurs in the girdle region in which three centers arising more or less simultaneously are usually distinguishable (Fig. 18). The center for the scapula is the first to appear. It grows in a dorso-ventral direction and gives rise dorsally to a broad supra-scapula, and ventrally fuses with the other two centers. The coracoid center is the next to appear, lying ventral to the scapula and posterior to the future procoracoid. It represents the ventral center of chondrification. The last to differentiate in the girdle formation is the procoracoid. It grows in an antero-ventral direction and forms the cephalic portion of the girdle. As soon as the centers of chondrification appear in these regions, they grow together and form a complete girdle. During the development of the girdle, the region which later gives rise to the glenoid cavity meets the opposed inward growth of the proximal epiphysis of the humerus and extends over it. Through this process of opposed growth, the head of the humerus grows in, and becomes closely applied to the girdle, forming the characteristic ball and socket joint (Figs. 23, 33). By examining successive stages during this period, one finds that the chondrification of the girdle takes place rather rapidly. Thus in a 16 mm. embryo, the girdle region is filled with undifferentiated cells, but about two days later when the embryo is 17 mm. in length, the rudiments of the three principal parts of the girdle with the glenoid cavity are already well outlined. In a slightly later stage, when the embryo is 18 mm. in length, the girdle is well along in the process of chondrification.

Following the appearances of the primordium of the girdle, the rudiments of ulna and radius differentiate respectively. At first they are two compact masses of cells with indistinct boundaries; but as soon as they are laid down, the process of chondrification proceeds rapidly. The earliest appearance of these structures is in an embryo of 17 mm. (Fig. 14), and when the embryo reaches 18 mm. in length, the ulna and radius have become definite cartilages (Fig. 16). By the time the embryo is 19 mm. long, they show the tendency to form three zones of cells along with the differentiation of diaphysis and epiphysis.

Subsequent to the differentiation of the ulna and radius, the elements in the carpal region become discernible (Figs. 15, 16). In spite of their

slightly later appearance, they are ultimately chondrified at the same time with the ulna and radius. In Necturus, the ulnare and intermedium, which are coalesced in later stages, arise as separate elements.

Simultaneously with the appearance of the carpal elements, four digits corresponding to the adult fingers are recognizable in the distal region of the limb (Fig. 14). They pass rapidly from the period of precartilage to the stage of active chondrification when the cartilages are formed. The duration of this process of development extends from the embryo of 17 mm. to the embryo 19 mm. in length.

Owing to the rapid development of the limb skeleton beginning in embryos 16 mm. long in which centers of chondrification just become distinguishable in the different parts of the limb, and the formation of the definite structures from all rudiments in embryos 19 mm. in length, close examinations have been made in the serial sections during this period with the view of determining whether or not there occur any variations from what is found in the adult limb. So far the writer has found that as soon as all primordia become discernible, they assume the essential characteristics of the adult organs and give no indication as to their phylogenetic conditions.

2. Histogenesis of Muscle

A. Development of the Myoblasts

The development of the limb muscle in Necturus maculosus takes place at the same stage with the differentiation of the primordial skeletal centers. The first indication of its development occurs in the embryo 15 mm. in length (Fig. 10). In this stage, the blood vessels are just arising in the limb region and the undifferentiated mesenchyme shows the tendency of formation into the peripheral and the axial masses. Through the process of rapid growth of the limb bud, the distinction between these two masses of cells becomes more conspicuous in the next stage. In a transverse section of a 16 mm. embryo, the mesenchyme cells in the axial mass as shown (Fig. 11) are arranged concentrically into a condensed skeletal core, whereas the cells in the peripheral region are separated into two dorsal and two ventral masses. The dorsal masses are distributed respectively as the proximal and the distal groups of cells. The proximal group is situated on the dorso-basal region of the limb bud, while the distal group occupies the outer peripheral surface of the axial core. The ventral masses also consist of the proximal and the distal groups of cells. The proximal group is located on the ventral base of the limb just opposite the proximal group of the dorsal mass, whereas the distal group is applied to the outer ventral portion of the

limb. There is no sharp line of distinction existing between the proximal and the distal groups, but a division is indicated by the presence of a loose mass of cells between them. The regions between the dorsal and ventral masses of cells and the axial skeletal core are separated respectively by the dorsal and the ventral branches of the spinal nerve. These early differentiated masses of cells have been traced as the premuscle tissues.

In this early stage of myogenesis, the cells in the condensed premuscle masses are not arranged in any definite direction. Some of the cells are still oval in shape, but many of them are rapidly undergoing the process of elongation. The myoblasts, when they are first recognizable, possess an elongated outline, distinct nuclear membrane, and clear chromatin network consisting of many karyosomes (Fig. 19). The myoblasts increase considerably in number chiefly by mitotic division, although the transformation of the undifferentiated mesenchyme into the myoblasts also takes place in the process of growth. During the rapid differentiation of the premuscle tissues, the syncytial cytoplasm at first is indistinct, but soon appears as an extensive anastomosis among the myoblasts (Fig. 19). After the myoblasts multiply, they continue to grow with the long axes of the nuclei and cell masses in the direction of the longitudinal growth of the primordial skeletal blastema.

B. Appearance of Fibrillation

In embryos 17 mm. in length, the myofibrillae arise from the syncytial cytoplasm at first without cross striations. They appear as homogeneous structures much as the cartilage matrix appears in the early stage. Upon close examination under high magnification, two kinds of fibrillae are detected, the coarse and the fine. The coarse fibrillae are slightly spiral in outline, mixed intermittently with the fine ones which are arranged more or less in parallel lines.

As to the formation and nature of myofibrillae, many hypotheses had been held by different investigators, but the modern workers generally agree that they are intra-cellular and are formed in the cytoplasm. This view is supported by the fact that just before the appearance of the fibrillae, the syncytial cytoplasm is densely interwoven among the myoblasts (Fig. 19). As soon as the fibrillae arise, the cytoplasmic processes disappear and are replaced by the fine strands known as myofibrillae (Fig. 20). According to the observation of Carey ('20, '21), in the case of the pig, the myofibrillae arise from the cytoplasm at first as parallel rows of isolated granules which later fuse to form the delicate strands.

C. Formation of Cross Striations

The first evidence of cross striations in the limb muscles of Necturus is seen in the embryo 19 mm. in length (Fig. 21). In the transformation of homogeneous myofibrillae into the striated muscle, some conspicuous changes take place in the nuclei of myoblasts. The nuclei are now much longer and more pointed. The length is several times the width and they are often tapered at both ends. The karyosomes are more numerous and larger than in the preceding stages. They are arranged in an irregular axial mass and a long peripheral band. Those in the axial mass are larger than those in the periphery, while the latter are closely applied to the nuclear membrane appearing as a continuous band. It is also noticeable that the nuclei are less numerous in the striated muscle than in the preceding stage. Simultaneously with the formation of cross striations, the sarcolemma arises to invest the myofibrillae, forming a distinct muscle fiber.

3. Development of the Spinal Nerve

In embryos 9 mm. in length, in which the anterior limb rudiment first becomes discernible, the spinal cord as seen in transverse sections is elongated, oval in outline and with its ventro-lateral sides slightly compressed (Fig. 27). The cells in the cord are somewhat elongated and are arranged radially with respect to the curvature of the cord as a whole. They have not yet reached to the outer limiting membrane which is separated from them by a zone of clear space, in which delicate cytoplasmic processes are often found. The cell walls are indistinct with numerous large yolk granules packed together. Some of the cells at the peripheral portion remain rounded in shape. At this period, the neural crest has segregated and migrated ventrally to the middle level of the spinal cord. The nuclei in the crest are usually oval with their long axes arranged in different directions. During the downward migration of the neural crest, the sclerotome cells arise in the region between the myotome and the notochord and eventually meet those of the crest. When the cells of these two sources come into close contact with one another, their difference in origin is hardly distinguishable, since they all possess the same staining character and general outline and are heavily laden with yolk granules. Similar conditions were also observed by Harrison ('24) in the case of the frog.

When the embryo is 10 mm. in length, the cells in the spinal cord have increased greatly in size and number. They are more elongated and arranged in transverse rows, and have extended to the outer limiting membrane. Numerous mitotic figures are frequently seen. The cells in

the neural crest also have begun to elongate in the direction of their growth and have reached the ventral end of the spinal cord. The nerve fibers are beginning to appear among the neural-crest cells and run closely to the motor nerve fibers just as the latter are issuing from the ventro-lateral border of the cord.

Embryos of a slightly later stage show a definite relation between the neural crest and the motor root. The neural-crest cells condense in compact masses forming the spinal ganglia with the segmental arrangement. The cells in the ganglia which are in active mitotic division, grow downward to the middle level of the notochord (Fig. 28). As the motor fibers grow out from the cord, they pass through the spinal ganglia and fuse with their fibers. The motor fibers grow downward and outward as slender stalks to the lower part of the myotome near the ventral level of the notochord. The nerve cells found in the motor fibers are spindle-shaped, showing variable relations in the different parts of the fibers. In the intra-medullary region of the motor nerve root, where the nerve fibers take their origin and penetrate through the wall of the cord, no cells are found. As soon as the motor fibers grow out from the outer limiting membrane, they are immediately surrounded by the ganglion cells. Examination of the spinal cord shows that there is no trace of outward migration of medullary cells along the motor root at this stage. The cells in the motor root are closely arranged in the same direction with those of the spinal ganglion and appear as one stream of cells. Evidently this suggests that the migration of cells from the spinal ganglion to the motor root takes place in embryos of this period.

In embryos 13 mm. in length, the spinal ganglia become more compact (Fig. 29). The motor nerves grow further downward and outward along the median margin of the myotome as far as the somato-splanchnopleuric angle. The yolk granules have now entirely disappeared both in the spinal cord and in the spinal ganglia.

Embryos of still a little older stage show that the motor nerves have passed out beyond the lower end of the myotome and reach the base of the limb bud (Fig. 10). The nerve trunks now become more fibrous and the cells are less numerous in their distal region.

When the embryo reaches 16 mm. in length, the motor roots have entered the limb region and divide into a dorsal and a ventral branch (Fig. 11). The dorsal ramus is shorter and goes to the dorsal premuscle mass, while the ventral ramus is longer and enters the ventral premuscle mass. Enclosed between them is the dense portion of the axial skeletal blastema. The motor fibers now increase in diameter and become slightly wavy with a few spindle-shaped cells scattered in the different parts of the nerve. From this stage onward, the nerves in the limb region

are undergoing further division and union in the formation of the brachial plexus.

V. ATTAINMENT OF ADULT CONDITIONS

1. Skeletal System

A. Necturus 19–27 mm.

The development of the appendicular skeleton has been traced in the previous sections up to the period at which all primordia have been laid down and are rapidly undergoing the process of further differentiation. After that period, the limb skeleton continues its growth toward the adult form. The details of its development are as follows:

a. The pectoral girdle

Scapula (Fig. 24).—The scapula is composed of a long and slender cartilage surrounded by a layer of perichondrium. It extends dorsally to the middle level of the notochord and turns slightly mesiad as it grows downward in the curvature of the body wall. The cartilage is thin and flattened laterally. Toward both ends, the scapula increases in size.

Supra-scapula (Fig. 25).—The supra-scapula is directly continuous with the scapula. At this period, it is composed of a condensed mass of cells which are in the process of active chondrification. It extends dorsally to the level of the ventral margin of the spinal cord. It is broader and thinner than the scapula and is surrounded by a condensed mass of mesenchyme cells.

Procoracoid (Fig. 17).—The procoracoid arises from the ventro-anterior portion of the girdle and grows cephalad as a long cartilaginous bar. It extends from the anterior margin of the third myotome to the posterior end of the fifth. Its cephalic end is surrounded by a mass of mesenchyme.

Coracoid (Fig. 18).—The coracoid is a broad, thin piece of cartilage forming the ventral portion of the girdle. Its anterior border elongates in the direction of the procoracoid and forms a reëntrant notch in the proximal region between the junction of the procoracoid and the coracoid. The cartilage grows downward toward the ventro-median line, and is surrounded by the undifferentiated mesenchyme. At this period the coracoids have not yet met in the ventro-median line.

Glenoid cavity (Fig. 18).—The glenoid cavity is well outlined with distinct cartilaginous ridges around the edge. It forms a sort of cap-like

structure which encloses the head of the humerus as the latter grows into it. The wall of the cavity including the ridges is relatively thin at this period.

b. The free limb

HUMERUS (Fig. 15).—The humerus consists of a shaft and two epiphyses. The shaft is long and slender, while the epiphyses are broad. The epiphyses have surfaces which articulate with the respective neighboring cartilages. The proximal epiphysis is semi-spherical in outline with its cells arranged crowdedly into a concentric form. It presses closely against the wall of the glenoid cavity. Toward its distal end it gradually narrows down and becomes imperceptibly fused with the shaft. The distal epiphysis is larger than the proximal one, and is now beginning to differentiate into the external and internal condyles with a shallow groove between them. The whole cartilage of the humerus inclines in a postero-ventral direction.

ULNA AND RADIUS (Figs. 16, 22).—The ulna and the radius are well formed in cartilage. The olecranon process is differentiated in the proximal part of the ulna to form a sigmoid notch which fits closely over the distal epiphysis of the humerus. The middle portion of the ulna, like the shaft of the humerus, is more slender than its ends. At the distal end, its articulating surface is dorso-ventrally flattened against the ulnare and a part of the intermedium. The radius enlarges at the proximal part with its dorsal surface slightly depressed to form a distinct socket which articulates with the ball-shaped condyle of the humerus. Toward the distal end, the radius also increases in size with its surface dorso-ventrally flattened and fitted into the lateral surface of the intermedium and dorsal surface of the radiale.

CARPUS (Fig. 22).—The carpal region consists of seven cartilages arranged in three rows. The proximal row includes the ulnare, the intermedium, and the radiale. The ulnare is separated from the intermedium in the early stage, but becomes coalesced at this period leaving only a foramen for the passage of a blood vessel. The middle row consists only of a small centrale situated in the center of the carpal region. The distal row is composed of three cartilages. Carpalia 1 is lost. Carpalia 2 articulates chiefly with the outer proximal surface of metacarpal II. Carpalia 3 covers the proximal surface of the metacarpal III and also extends toward the inner proximal surface of metacarpal II. Carpales 4 and 5 are fused to form one cartilage which articulates with the proximal ends of metacarpals IV and V.

DIGITS (Fig. 22).—The digits are four in number, the first one being

lost. The segmentation in the digits is first seen in the embryo 18 mm. in length. They are separated into distinct phalanges in embryos of 19 mm. There are nine phalanges in the digits with three in the fourth digit and two in each of the others.

There are no joint cavities between the cartilages in the distal part of the hand at this period, but each cartilage is distinctly demarcated by its deeply stained peripheral matrix.

B. Necturus 30–39 mm.

The appendicular skeleton of the larva at this period has become entirely cartilaginous and has practically attained the conditions which are comparable with the adult state, except that the scapula of the girdle and the long cartilages of the free limb have not yet become ossified. One must keep in mind, however, that even in the adult, a large proportion of the entire skeletal system remains an unossified cartilage.

a. The pectoral girdle

Scapula (Fig. 31).—The scapula increases greatly in size and extends farther dorsally than in the preceding stage. It becomes flattened laterally and grows broad antero-posteriorly. Its peripheral surface, as seen in transverse sections, does not contain any undifferentiated condensed mesenchyme. It is lined by a layer of thick cartilage matrix which stains dark blue with picro-indigo-carmine at this period. The cells in the cartilage are usually polyhedral in outline, with rounded nuclei, large lacunae, and heavily thickened capsules.

Supra-scapula (Fig. 3).—The dorsal surface of the scapula broadens out into a hatchet-shaped cartilage known as the supra-scapula. It extends dorsally as far as the transverse processes of the vertebrae. There is no distinct division between the scapula and the supra-scapula in the larval stage, but they are clearly distinguishable in the adult where the scapula becomes ossified, and the supra-scapula remains cartilaginous throughout life. In transverse sections, the supra-scapula and the scapula are seen to cover almost the full extent of the lateral body wall.

Procoracoid (Fig. 33).—The procoracoid can be studied best from the frontal sections where the full extent of the structure can easily be seen. It is the longest cartilage in the girdle. Its anterior tip extends to the level of the distal end of the fifth visceral arch. The cartilage at this period is precisely similar to the adult form. It is relatively broad at the base but immediately becomes narrow as it grows forward. The cartilage enlarges a little beyond the mid-region, taking the form of a scalpel blade with its blunt tip at the free end. The edge of the blade is directed toward the ventro-median line.

CORACOID (Fig. 34).—The coracoid is much larger than in the preceding period. It grows rapidly toward the ventro-median line and soon extends beyond it, overlapping its fellow from the opposite side. The left coracoid is usually the ventral or superficial one where the overlapping occurs. Each cartilage appears as a circular flat disc with a deep reëntrant angle near the proximal region of the procoracoid. Between the reëntrant angle and the glenoid cavity, there is a foramen through which the supra-coracoid nerve passes to supply the muscles on the ventral portion of the girdle.

GLENOID CAVITY (Fig. 32).—The glenoid cavity is on the dorsal portion of the coracoid. It is now much enlarged and surrounded with thickened cartilage which becomes elevated into a strong ridge on the external surface, forming a sufficiently deep socket for the reception of the humerus. There are also some ligamentous tissues, as seen in the adult limb, growing out from the cartilaginous ridge and becoming attached around the proximal epiphysis of the humerus. This strengthens the attachment of the free limb to the girdle.

The whole pectoral girdle now appears as a thin piece of cartilage consisting of three lobes with a cavity in the center. From this cavity, there grows out dorsally the slender scapula, which broadens out at its free end into a hatchet-shaped cartilage known as the supra-scapula. On the anterior margin of the cavity, the procoracoid grows out in an antero-ventral direction as a long, thin piece of cartilage resembling the blade of a scalpel. Below the cavity, the cartilage extends ventrally as a flat rounded plate forming the characteristic coracoid found in the urodeles.

b. The free limb

HUMERUS (Fig. 33).—The humerus has much the adult shape although it has not yet become ossified. The head is ball-shaped and fits into the glenoid cavity of the shoulder girdle. Toward the ventral surface of the head, the cartilage becomes flattened laterally and prolonged into a sharp ridge which gradually recedes toward the shaft. This ridge is known as the crista ventralis (Wilder, '03) and serves for the insertion of most of the ventral shoulder muscles. The humerus now is a cylindrical cartilage. It gradually enlarges toward its distal end and is imperceptibly fused with the distal epiphysis. At this period, the differentiation of the external (lateral) and the internal (median) condyles at the distal epiphysis becomes more advanced. The external condyle, which is larger and rounded in outline, fits into the depression of the head of the radius. It also serves as a point of origin for the extensor muscles of the forearm and hand. The internal condyle is less prominent and does not take part directly in the formation of the elbow

joint, though it gives origin to the flexor muscles of the forearm and hand. The groove between the two condyles articulates with the sigmoid notch and olecranon process of the ulna and also with the head of the radius.

ULNA AND RADIUS.—The ulna and radius have the same form as that found in the adult. The ulna is comparatively slender with nearly uniform thickness throughout. Its proximal end greatly elongates dorsally into an olecranon process with a prominent sigmoid notch. Its distal end becomes slightly enlarged and articulates chiefly with ulnare. The radius is slightly thicker than the ulna, and its shaft and epiphyses are quite distinct from one another. The proximal epiphysis gives rise to a sort of socket-like structure which receives the external condyle of the humerus. The distal epiphysis is laterally flattened with a slight acute apex toward the median line. It articulates with the intermedium and the radiale.

CARPUS.—The cartilages that constitute the wrist increase in size slightly and move a little apart from one another, the narrow spaces between them becoming filled with condensed cells. The arrangement of the carpal cartilages follows the same plan as that found in the adult, and is similar to the conditions described in the preceding section.

DIGITS.—The digits remain the same as in the preceding period, in regard to their number and general arrangement. Each cartilaginous segment is now differentiated into a slender shaft and has a slightly enlarged epiphysis at each end, with the exception of the terminal phalanx which has no epiphysis at the distal end.

As a whole the appendicular skeleton of Necturus maculosus is relatively weak in comparison with that of the higher forms, correlated with the fact that the locomotion of the animal does not depend primarily upon the limbs, but rather upon the undulating movement of the caudal fin and the entire body of the animal.

2. MUSCULAR SYSTEM

The limb muscles, as mentioned in previous sections, first arise as two dorsal (dorso-proximal and dorso-distal) and two ventral (ventro-proximal and ventro-distal) groups of condensed mesenchyme cells at the 16 mm. stage. After that stage, the premuscle masses, through processes of separation and differentiation, gradually give rise to the muscles, which grow toward the adult conditions.

A. Necturus 17.5 mm.

a. Dorso-proximal muscle mass

In embryos of this stage, several muscles have developed from this mass of premuscle, namely, latissimus dorsi, dorsalis scapulae, anconeus scapularis, and anconeus coracoideus.

LATISSISMUS DORSI (Fig. 53).—This arises as a small muscle posterior to the dorsal portion of the cartilaginous scapula. It is connected with the posterior border of the dorsalis scapulae by a mass of condensed cells. Its origin and insertion have not yet been differentiated.

DORSALIS SCAPULAE (Fig. 52).—This covers the lateral surface of the scapula extending downward from the dorsal part of the cartilage, and becomes fused at its distal end with the procoraco-humeralis near the junction between the scapula and the procoracoid.

ANCONEUS CORACOIDEUS (Fig. 53).—This arises from the posterior tuberosity of the coracoid just posterior to the glenoid cavity, and runs as far as the distal third of the humerus, where it blends with muscle fibers of anconeus scapularis.

ANCONEUS SCAPULARIS (Fig. 53).—This lies chiefly on the dorsal surface of the humerus and extends from the proximal half of the cartilage to its distal end. At this stage, there is no distinct point of insertion, since the olecranon process of the ulna has not yet become differentiated.

b. Dorso-distal muscle mass

In embryos of this stage, two masses of condensed cells are discernible on the dorsal flexor surface. One is in the antebrachial region and the other in the carpal portion. They are more or less united, and have not yet differentiated definitely into the separate extensor muscles.

c. Ventro-proximal muscle mass

Several muscles have been developed from the premuscle cells of this group. They are the pectoralis, supra-coracoideus, procoraco-humeralis, coraco-brachialis longus, and humero-antebrachialis.

PECTORALIS (Fig. 52).—At this stage, this muscle arises posterior to the supra-coracoideus and partly overlaps its posterior border. It lies on the ventral side of the body posterior to the coracoid and becomes inserted upon the ventral aspect of the head of the humerus.

SUPRA-CORACOIDEUS (Fig. 52).—This appears at this stage as a broad sheet of muscle covering the ventro-proximal part of the coracoid, and follows the general outline of the cartilage. Its posterior border is overlapped by the pectoralis. It becomes inserted upon the ventral aspect of the proximal epiphysis of the humerus adjacent to the point of insertion of the pectoralis.

PROCORACO-HUMERALIS (Fig. 52).—This arises as a small mass of fibers running longitudinally with the cartilaginous procoracoid. It covers the proximal half of the ventral surface of the cartilage, and becomes

fused with the ventral end of the dorsalis scapulae at the junction between this cartilage and the scapula.

CORACO-BRACHIALIS LONGUS (Fig. 53).—This muscle lies on the median surface of the flexor side of the humerus. In a reconstruction of the limb at this stage, it is seen arising from the posterior tuberosity of the coracoid, just posterior to the glenoid cavity. It extends as far as the distal part of the humerus, where it comes into contact with a group of condensed cells, which are later to become differentiated into the flexor muscle.

HUMERO-ANTEBRACHIALIS (Fig. 52).—This muscle and the coraco-brachialis longus are the two long muscles situated on the flexor side of the humerus. They correspond to the biceps of higher forms. The former lies upon the lateral side, and the latter upon the median aspect. In embryos of this stage, the humero-antebrachialis appears as a small mass of fibers covering the lateral portion of the flexor surface of the humerus. It extends from the proximal half of the cartilage to the distal end. Its origin and insertion are not clearly differentiated at this stage.

d. Ventro-distal muscle mass

At this stage, the distal part of the flexor surface of the limb is covered by condensed masses of cells which later give rise to the definite flexor muscles.

B. Necturus 19 mm.

In embryos of this stage great progress has been made in the differentiation of the muscles of the dorso-distal and ventro-distal muscle masses. The superficial layers of the extensor and the flexor muscles first become distinguishable, and the dorso-proximal and the ventro-proximal masses increase in size.

a. Dorso-proximal muscle mass

LATISSIMUS DORSI (Fig. 55).—There is no great change in this muscle at this stage. It still appears as a muscular mass growing out posterior to the dorsalis scapulae, extending dorsally and ventrally for a short distance, and ending abruptly without reaching any cartilage.

DORSALIS SCAPULAE (Fig. 54).—This increases in size and grows dorsally to the ventral margin of the rudimentary supra-scapula. It extends beyond the posterior border of the cartilage, leaving the anterior margin of the cartilage free from the muscular attachment. The distal end, as seen in the preceding stage, is fused with the procoraco-humeralis.

ANCONEUS HUMERALIS LATERALIS (Fig. 54).—This muscle lies on the lateral surface of the humerus and belongs to the same group as the anconeus scapularis and the anconeus coracoideus mentioned previously. It arises from the proximal epiphysis of the humerus, and extends obliquely along the lateral surface of the cartilage. Its fibers become fused at their distal part with the anconeus scapularis on the dorsal surface, and insert with that muscle upon the olecranon process of the ulna.

b. Dorso-distal muscle mass

Three muscles covering the extensor surface of the forearm and hand are recognizable at this stage. They are the humero-metacarpalis, extensor radialis, and the extensor ulnaris.

HUMERO-METACARPALIS (Fig. 54).—This muscle arises from the lateral condyle of the humerus and extends over almost the entire dorsal surface of the forearm to the basal margin of the digits.

EXTENSOR RADIALIS (Fig. 54).—This muscle lies on the radial side of the forearm, and entirely covers the dorsal surface of the radius. Toward the median part of the muscle, the fibers blend more or less with those of the humero-metacarpalis.

EXTENSOR ULNARIS (Fig. 54).—This muscle occupies the marginal border of the ulna and is fused dorsally with the humero-metacarpalis at this stage. It arises from the external condyle of the humerus along the margin adjacent to the olecranon process of the ulna, and becomes attached upon the lateral border of the ulna.

c. Ventro-proximal muscle mass

There is little differentiation in the muscles of this group at this stage. Besides their general growth in size, they have made no remarkable changes.

d. Ventro-distal muscle mass

The muscles that first become recognizable as the distinct flexor muscles at this stage are the palmaris superficialis, flexor ulnaris, and flexor radialis. They are more or less fused with each other.

PALMARIS SUPERFICIALIS (Fig. 55).—This is a broad mass of muscle covering the flexor surface of the antebrachium. It arises from the median condyle of the humerus between the radial and the ulnar flexors, and extends to the base of the digits.

FLEXOR ULNARIS (Fig. 55).—This muscle lies upon the flexor aspect of the entire ulna and is partly fused with palmaris superficialis. Its origin and insertion have not yet become definitely differentiated.

FLEXOR RADIALIS (Fig. 55).—This appears as a short muscle on the radial side of the palmaris superficialis, and is partly fused with the latter. It arises from the radial side of the medium condyle of the humerus, and inserts upon the outer margin of the radiale.

C. Necturus 21-39 mm.

In embryos of this period, the appendicular muscles are growing toward the conditions which are comparable with those of the adult limb. The muscles in the distal part of the limb have separated into the superficial and the deep layers. Several muscles which are not developed in the girdle and upper-arm at the preceding stages are now differentiated.

a. Dorso-proximal group of muscles

LATISSIMUS DORSI (Figs. 37, 56-59).—This muscle at this time is broad and fan-shaped, and covers the lateral side of the body between the fifth and eighth myotomes. It extends much farther dorsally than the supra-scapula, and gradually narrows down toward its ventral end where it is inserted into the anterior lateral lip of the glenoid cavity by the ligamentous fibers, as seen in the adult.

DORSALIS SCAPULAE (Figs. 37, 56-59).—During the further growth of the supra-scapula in embryos of this period, the dorsalis scapulae also increases greatly in length. It now originates near the free margin of the supra-scapula, and runs somewhat obliquely along the scapula. Hence the muscle is free from the distal half of the cartilage and converges to insert into the lateral surface of the proximal epiphysis of the humerus.

TRAPEZIUS (Figs. 37, 56-59).—This muscle appears slightly later than those mentioned above. It becomes distinguishable first in the embryo of 21 mm. At this stage, it is seen on the anterior margin of the cartilaginous scapula. It is broad at the dorsal end, but gradually becomes narrow as it grows downward toward the middle of the scapula. In embryos between 27 mm. and 39 mm., the muscle begins to grow in both the dorsal and ventral directions, as in the case of the latissimus dorsi. Dorsally it grows into a broad, thin sheet between the second and fourth myotomes, and extends beyond the dorsal margin of the supra-scapula. Ventrally it converges gradually into a narrow strip along the anterior margin of the scapula, and becomes inserted at the distal end of the cartilage. The dorsal part of the muscle is covered laterally by the levator arcum and dorso-laryngeus.

OMOHYOIDEUS (Fig. 37).—This muscle is seen best in sagittal sections. In embryos between 21 mm. and 23 mm., it blends more or less

with the visceral muscles, and can hardly be distinguished as a separate muscle. In embryos 34 mm. to 39 mm., it appears as a narrow muscle situated antero-ventrally to the trapezius. Its fibers arise from the third epibranchial arch, and run obliquely from an antero-dorsal to a postero-ventral direction, inserting into the deep notch between the scapula and the procoracoid. Its dorsal part is overlapped laterally by the dorso-laryngeus.

LEVATOR ANGULI SCAPULAE (Figs. 38, 56-58).—The first appearance of this muscle occurs in the embryo 21 mm. in length. At this stage, it is a short, narrow muscle arising anteriorly to the supra-scapula, and becoming inserted at its anterior margin. In embryos 27 mm. to 39 mm., it increases greatly in length, and appears as a long narrow band extending horizontally between the third epibranchial arch and the anterior margin of the supra-scapula. It is covered laterally by the levator arcuum, dorso-laryngeus, and trapezius. Its point of insertion is at the inner margin of the anterior border of the supra-scapula. In the adult, the muscle arises from the posterior end of the exoccipital as a fine tendon which broadens into fleshy fibers toward the supra-scapula. This tendenous tissue is not yet differentiated in the larval stage.

SERRATUS ANTERIOR (Figs. 38, 56-58).—In the embryo 21 mm. in length, this muscle is composed of a small mass of fibers lying on the median side of the supra-scapula, and extending from the fifth to the seventh myotomes. It is entirely covered laterally by the latissimus dorsi. When the embryo reaches 27 mm. in length, the muscle increases in length posteriorly, and also extends farther anteriorly along the inner surface of the supra-scapula to the region near to the insertion of the levator anguli scapulae. The muscle arises as a narrow strip posterior to the supra-scapula and gradually broadens and separates into several strips near its insertion upon the median surface of the cartilage.

SCAPULO-HUMERALIS (Figs. 36, 57).—This muscle first arises in the embryo 21 mm. in length as a narrow band of muscle extending from the deep sinus between the scapula and the tuberosity of the coracoid to the proximal part of the humeral shaft. In the 27 mm. embryo, the muscle enlarges near its point of origin, and runs distally between anconeus scapularis and anconeus coracoideus toward the humerus. It inserts upon the inner surface of the mid-region of the cartilaginous shaft.

ANCONEUS SCAPULARIS (Figs. 35, 57, 58).—This is a large muscle covering the dorsal surface of the humerus. It arises from the lateral edge of the glenoid cavity as ligamentous fibers which are continuous with the insertion of latissimus dorsi. Thus the origin of the former appears to be the insertion of the latter.

ANCONEUS HUMERALIS LATERALIS (Fig. 35).—This is a narrow band of muscle running obliquely along the lateral surface of the humerus. It arises from the proximal end of the cartilaginous shaft, and extends obliquely toward the distal end of the cartilage. It blends with the anconeus scapularis at the insertion upon the olecranon process.

ANCONEUS CORACOIDEUS (Figs. 35, 56-58).—This muscle covers the median surface of the humerus, and extends somewhat obliquely from the posterior tuberosity of the coracoid to the distal half of the humerus, where it fuses with the median edge of the anconeus scapularis.

ANCONEUS HUMERALIS MEDIALIS (Fig. 35).—This is a deep muscle closely applied to the entire dorsal surface of the shaft of the humerus. It is overlapped by the anconeus scapularis and anconeus humeralis lateralis, but is separated from them by large blood vessels which pass between them.

b. Dorso-distal group of muscles

HUMERO-METACARPALIS (Fig. 42).—In the embryo of 21 mm., this muscle appears as a large triangular mass. Toward the distal end, it separates into three strips which end in the interdigital spaces between the metacarpals.

EXTENSOR RADIALIS SUPERFICIALIS (Fig. 41).—This is the superficial strip of the extensor radialis. It arises from the external condyle of the humerus, and lies along the free margin of the radius. The deep fibers of this muscle become attached to the radius in their course to the insertion upon the radiale and carpalia 2. The muscle can be seen superficially from the lateral aspect of the radius.

EXTENSOR ULNARIS (Fig. 41).—At this period, this appears as a distinct, separate muscle, although the general outline remains the same as in the preceding stage.

EXTENSOR RADIALIS PROFUNDUS (Fig. 41).—This muscle is covered by the humero-metacarpalis and is situated medial to the extensor radialis superficialis. Its point of origin is at the ventral end of the external condyle, being distal to that of the superficialis. The muscle is inserted along the ulnar aspect of the radius.

SUPINATOR (Fig. 41).—This is a short muscle running obliquely in the carpal region and is first seen in the embryo of 22 mm. A great part of this muscle is covered superficially by the humero-metacarpalis, and only its distal portion can be seen from the radial margin of the latter muscle. It arises from the radial side of the ulnare-intermedium and extends obliquely to the lateral side of the proximal part of metacarpal II.

EXTENSOR BREVIS (Fig. 60).—This consists of four narrow strips of muscle which arise from the distal margins of the carpales, becoming distinguishable in the embryo 21 mm. in length. The first and second strips have their points of origin at the carpales 2 and 3, and the third and fourth at the carpalia 4 + 5. They lie along the dorso-median surface of the digits and insert upon the bases of the terminal phalanges.

c. Ventro-proximal group of muscles

PECTORALIS (Figs. 39, 57-59).—In the early stages (17.5-19 mm.) this muscle arises posteriorly to the supra-coracoideus and partly overlaps its posterior end. The two are somewhat connected by a mass of condensed cells. In embryos between 27 mm. and 39 mm., the muscle extends further postero-ventrally toward the linea alba, and becomes a large sheet of muscle covering an extensive area between the coracoid and the ninth myotome. Its anterior part is still closely applied to the posterior part of the supra-coracoideus. These two muscles run in the same direction, and can be mistaken readily for a continuous piece of muscle. In transverse sections through the pectoral region, it is seen that the pectoralis on each side of the body grows rapidly toward the ventro-median line, and finally comes to lie beside the linea alba. In frontal sections, the fibers of the pectoralis are shown to be growing in various directions. The most anterior fibers at the level of the mid-region of the coracoid, incline slightly postero-laterally. Those just behind the posterior margin of the coracoid are arranged at right angles to the linea alba, while those in the middle part of the muscle run obliquely. Toward the distal part of the muscle, the fibers lie nearly longitudinal. From their extensive origin along the linea alba, the muscle fibers converge toward the region between the proximal part of the humerus and the coracoid, and finally insert upon the crista ventralis of the humerus.

SUPRA-CORACOIDEUS (Figs. 34, 39).—The general outline of this muscle remains the same as that in the preceding stages. Its insertion becomes clearly differentiated upon the crista ventralis just proximal to the insertion of the pectoralis.

PROCORACO-HUMERALIS (Figs. 33, 59).—During this period, the muscle increases both in length and width. It grows further anteriorly, and extends from the lateral edge to the median edge of the procoracoid. It is inserted upon the crista ventralis of the humerus. While this muscle is growing, the rectus superficialis hypobranchialis posterior also becomes differentiated from the trunk muscles. The latter has an extensive origin anterior to the procoracoid, and grows posteriorly over its free end, becoming fused with the anterior part of the procoraco-humeralis on the dorso-lateral side of the cartilage.

RECTUS SUPERFICIALIS HYPOBRANCHIALIS POSTERIOR (Figs. 57-60).—This muscle becomes distinguishable in the embryo 21 mm. in length. At that stage, the muscle appears as a single strip arising anterior to the free end of the procoracoid. It begins to separate into the external (lateral) and the internal (median) strips as it enters the cartilage. The external strip is partly fused with the procoraco-humeralis on the lateral side of the procoracoid. The internal strip is closely applied to the distal part of the median surface of the cartilage. These two strips are partially connected with each other on the dorsal edge of the cartilage between the lateral and the median surfaces. In embryos between 34 mm. and 39 mm., the separation between the lateral and the median strips becomes more distinct. They originate as a single band of muscle from the posterior end of the first ceratobranchial arch, but soon separate as they grow posteriorly toward the cartilaginous procoracoid. The median strip now arises from the anterior end of the cartilage, and grows along its median margin for a long distance, finally converging into a narrow insertion upon the external surface of the girdle just anterior to the glenoid cavity. The lateral strip courses along the lateral side of the procoracoid and partly fuses with the procoraco-humeralis as it inserts into the notch between the distal end of the scapula and the proximal base of the procoracoid.

CORACO-BRACHIALIS LONGUS (Figs. 37, 56-58).—This muscle now becomes separated from the anconeus coracoideus. It arises from the posterior tuberosity of the coracoid and inserts upon the distal half of the shaft of the humerus.

CORACO-BRACHIALIS BREVIS (Figs. 36, 37).—This is a small, deep muscle covered medially by the coraco-brachialis longus, and laterally by the supra-coracoideus. It first becomes discernible in the embryo 21 mm. in length, and attains the adult condition at 27 mm. It arises from the posterior margin of the coracoid just ventral to the tuberosity, and extends slightly dorsally along the median aspect of the humerus, becoming inserted upon the proximal half of the cartilaginous shaft.

d. Ventro-distal group of muscles

PALMARIS SUPERFICIALIS (FIG. 56-58).—This muscle increases greatly in size, and extends distally to insert into the proximal margin of the palmar fascia. The latter arises as an aponeurotic sheet from the distal margin of the palmaris superficialis and reaches to the base of the metacarpals, where it separates into four tendon-like strips. They lie along the palmar aspect of the four digits, and finally become inserted upon the bases of the terminal phalanges.

FLEXOR ULNARIS (Fig. 40).—This now becomes a distinct muscle, and lies upon the entire flexor aspect of the ulna. It originates from the median condyle of the humerus adjacent to the olecranon process, and extends distally along the outer margin of the ulna. The fibers converge into a narrow insertion upon the lateral aspect of the ulnare-intermedium and the carpalia 4 + 5.

FLEXOR RADIALIS (Figs. 40, 56-58).—The general outline remains the same as in the preceding period. This appears as a distinct separate muscle without any fusion with the palmaris superficialis.

PALMARIS PROFUNDUS (Fig. 40).—This belongs to the deep series of muscles, and is covered externally by the palmar fascia. It arises from the radio-flexor aspect of the ulna, and runs across the surface of ulnare-intermedium. It is attached to the inner surface of the palmar fascia.

ULNARICARPALIS (Fig. 40).—This is first seen in the embryo of 21 mm. It is a narrow muscle situated between the palmaris profundus and the pronator. The fibers run longitudinally from the radial aspect of the ulna, extending downward to carpalia 4 + 5.

PRONATOR (Fig. 40).—This muscle is directly opposite the supinator on the extensor surface. The fibers run obliquely from the middle of the shaft of the ulna to the radial margin of the base of metacarpal II. The distal part of the muscle can be seen from the radial side of the palmar fascia.

CARPO-METACARPALIS (Fig. 41).—This consists of a group of deep muscles extending from the carpus to the metacarpals. They arise from the distal margins of carpales and insert into the distal end of the metacarpals. Two strips of the muscle are found on each metacarpal, one of which lies on the radial side, and the other on the ulnar side. In digit II, both the radial and ulnar strips arise from the distal margin of carpalia 3 and become attached to the ulnar border of metacarpal II. In digit III, the radial strip arises from carpalia 3, and the ulnar from the interspace between carpalia 3 and carpalia 4 + 5. In digit IV, both the radial and the ulnar strips originate from carpalia 4 + 5. In digit V, the radial and the ulnar fibers are larger than those found in the other digits. The radial strip arises from the ulnar half of the margins of carpalia 4 + 5 and inserts upon the radial margin of metacarpal V, while the ulnar strip grows out chiefly from the ulnar side of the distal margin of the ulnare-intermedium, and becomes attached to the ulnar margin of metacarpal V.

INTERMETACARPALES (Fig. 42).—There are three of these small muscles in the interdigital spaces in the deep region of the hand. These muscles appear somewhat triangular in shape and extend somewhat obliquely between the metacarpal cartilages.

In order to avoid confusion in using the terminology of the pectoral musculature of Necturus, a list of synonyms of the pectoral muscles is given here:

Mivart ('69)	*Wilder* ('12)	*Adams* ('26)
Latissimus dorsi	Latissimus dorsi	Latissimus dorsi
Deltoid	Dorsalis scapulae	Dorsalis scapulae
Trapezius	Trapezius	Cucullaris
Omohyoid	Omohyoideus	Omohyoid
Levator anguli scapulae	Levator anguli scapulae	Levator scapulae
Serratus magnus	Serratus anterior	Serratus magnus
Pectoralis	Pectoralis	Pectoralis
Subclavius	Procora-humeralis	Procoraco-humeralis
Subclavius ? (Part of above)	Rectus superficialis hypobranchialis posterior	Rectus superficialis hypobranchialis posterior
Triceps	Anconeus	Triceps (Quadriceps)
Biceps	Humeroantibrachialis	Biceps
Coraco-brachialis (1st strip)	Supracoracoideus	Supracoracoid
Coraco-brachialis (2nd strip)	Coraco-brachialis longus	Coraco-brachialis
.....	Coraco-brachialis brevis	Coraco-brachialis brevis
Subscapularis	Scapulo-humeralis	Scapulo-humeralis
Extensor longus	Humerometacarpalis	Extensor metacarpalis
Supinator longus	Extensor radialis superficialis	Extensor radialis
Ulnaris	Extensor ulnaris	Extensor ulnaris
Extensor brevis	Supinator	Supinator
.....	Extensores breves	.....
Pronator quadratus ?	Extensor radialis profundus	.....
Flexor longus	Palmaris superficialis	Palmaris superficialis
Pronator teres (2nd strip) ?	Palmaris profundus	.....
Flexor brevis	Pronator	Pronator
.....	Ulnaricarpalis	Ulnaricarpalis
Pronator teres (1st strip)	Flexor ulnaris	Flexor ulnaris
.....	Flexor radialis	Flexor radialis
.....	Carpo-metacarpales	.....
.....	Intermetacarpales	.....

3. Brachial Plexus

In order to get a clear picture of the brachial plexus in Necturus maculosus during the developmental period, a wax reconstruction of the brachial plexus in a larva of 34 mm. in length was made (Figs. 61-63). The plexus has attained the adult form at this stage. The brachial plexus is formed from the branches of the ventral roots of the spinal

nerves III, IV, and V, the spinal nerve IV forming the chief trunk (Fig. 30). The details of these nerves are as follows:

SPINAL NERVE III.—As soon as this nerve comes out from the spinal cord, it grows posteriorly toward the limb region. Before it enters the plexus, it separates into three branches. The anterior branch is small and is distributed to the body wall anterior to the girdle. The middle one is long and slender. It passes through the foramen between the glenoid cavity and the reëntrant angle of the coracoid forming the supracoracoid nerve. It innervates the muscles on the ventral portion of the girdle. The posterior branch is slightly larger than either of the first two. It joins the spinal nerve IV forming the plexus.

SPINAL NERVE IV.—This is the largest branch of the plexus, both in the larva and in the adult, and runs along the posterior margin of the scapula. At the distal part of this cartilage, the nerve anastomoses with the posterior ramus of the spinal nerve III forming the plexus.

SPINAL NERVE V.—This is the most posterior nerve contributing to the formation of the brachial plexus. It goes anteriorly, and just before reaching the plexus, it gives rise to two rami: the anterior ramus joins the spinal nerve IV in the plexus, while the posterior one is distributed to the body wall posterior to the girdle.

VI. EXTERNAL DEVELOPMENT OF THE LIMB

The foregoing descriptions are confined to the development of the internal structures of the anterior limb. A brief account concerning the development of the external form of the appendage may be given as follows:

The first indication of the external appearance of the limb bud is in an embryo 13 mm. in length. At this stage, the bud projects dorsally (Fig. 43) and measures about .5 mm. in length. In a slightly later stage, when the embryo is 15 mm. long (Fig. 44), the limb bud increases in size and measures about .8 mm. in length. It now inclines in a dorso-posterior direction at an angle of 45° to the long axis of the embryo. When the embryo reaches 16 mm. in length, the inclination of the limb bud increases to 75° (Fig. 45). It still bears no indication of separation into digits.

The first evidence of the digits occurs when the embryo is 17 mm. in length (Fig. 46). At this stage, the limb bud grows in a postero-ventral direction and has three digits. These are the second, third, and fourth fingers. In Necturus, the first one is lost and does not reappear during the ontogenetic development. From the examination of sections, it is

found that the rudiments of all four fingers (2nd, 3rd, 4th, and 5th) appear at the same stage, although their external indications are not shown simultaneously.

In an embryo of 18 mm., the limb bends and grows farther ventrally (Fig. 47). The convex aspect of the bending is directed dorsally, and the concave aspect toward the ventral side. At this stage also, the fifth finger becomes visible. Soon after the bending takes place in the limb, the division between the upper arm and the antebrachial region begins to become distinguishable. This division is noticeable in an embryo 19 mm. in length (Fig. 48). At this stage, the four fingers are stout and have blunt tips.

In the subsequent stages (20-33 mm.), the free limb increases in size and gradually assumes the adult features (Figs. 49-51). The upper arm grows nearly longitudinally to the long axis of the body, while the forearm and hand develop in a postero-ventral direction at an angle of about 45° to the upper arm.

VII. DISCUSSION

1. Fate of the Myotome Processes

Considerable work has been done on the relation of the myotomes to the early development of the limb in vertebrates. The results obtained, however, vary with the different investigators. Thus far there are two views regarding the fate of the myotome processes: (1) that the myotomes or their processes take part in the formation of the limb; (2) that the myotomes are in no way concerned in the formation of the limb, which arises as a thickening of the somatopleure.

According to Goette ('75), the limb muscles in Bombinator develop from the outer layer of the muscle plate. Jordan ('88), in his paper on the development of the anterior limb in Anura, accepts the viewpoint set forth by Goette. Kaestner ('93) believes that the myotomes take part in the formation of the limb in Anura at a very early stage. Field ('94) also described the limb muscles as being derived from the myotome processes in both urodeles and anurans. Mollier ('95) observed the growth of the muscle buds from the myotomes to the limb bud and their transformation into the limb muscles in Lacertilia. Later Tschernoff ('07) through his study on the development of the hind limb in the frog, concluded that the primordium of the limb is formed partly from the cells proliferated from the myotomes and partly from those derived from the somatopleure.

On the other hand, Paterson ('88), in his work on the fate of the muscle plate in the chick, thought that the muscle plate does not pass into the limb region, but is later transformed into the longitudinal muscles of the trunk. The limb muscles, he thought, are formed by the differentiation of the mesenchyme cells which form the primitive limb bud. Harrison ('95), in his study on the development of fins in teleosts, found that the ventral myotome processes in the region of the pectoral fin take no part in the formation of the muscles in the fin, but are differentiated into the coraco-hyoid muscle. The structures of the pectoral fin, including the cartilages and muscles, according to Harrison, are differentiated from the mesenchyme cells which develop from the somatopleure. Similar conditions were also described in his later work ('18), on the development of the fore limb in Amblystoma, in which he found that the limb develops as a thickening of the somatopleure in the region between the third and fifth myotomes. Byrnes ('98), through both her observation and experimental work on the development of the limb in urodeles and anurans, confirmed the results obtained by Harrison, and concluded that the ventral myotome processes in Amphibia give rise to the ventral muscles of the body wall, and take no part in the formation of the limb. Lewis ('10) proved by his experimental work that the limb in Amblystoma can develop normally in the absence of the myotomes in the limb region. His results were later confirmed by Detwiler ('18).

On the basis of the evidence of the present work on Necturus maculosus, the writer agrees with the viewpoint set forth by the second group of investigators that the limb arises as a thickening of the somatopleure, and is in no way derived from the myotomes or their processes. This conclusion is justified on the following grounds:

(1) The primordial anterior limb bud is found as a thickening of the somatopleure before the appearance of the ventral myotome processes in an embryo of 9 mm.

(2) The proximity of the limb bud and the myotome processes is merely due to the excessive growth of the pronephric tubules, which press the processes closely against the median side of the limb bud.

(3) During their close contact with the limb bud, the myotome processes are distinguished from the surrounding mesenchyme cells by their dorso-ventrally elongated nuclei and by their well-defined general form. No indication of a migration of cells from the myotome processes to the limb bud has been found.

(4) When the ventral processes are cut off from their respective myotomes, they grow downward along the outer border of the coelom, and later transform definitely into the ventral muscles of the body wall.

2. Formation of the Limb Skeleton

The early development of the limb skeleton in Necturus is quite similar to that of higher forms. Carey ('21), in his work on the genesis of bone and muscle in the pig, found that the primordial skeletal center in the limb bud arises by condensation of the undifferentiated mesenchyme cells. He also observed that from this center of condensation, the growth of the limb skeleton takes place in a proximo-distal direction. In Necturus, similar conditions also occur in the formation of the limb skeleton. Soon after the mesenchyme cells in the limb bud divide into the peripheral and the axial masses in the 16 mm. embryo, the chondrification of the cells in the axial mass immediately takes place. This is followed first proximally by the appearance of the rudiments of the girdle, and then distally at a slightly later stage by the differentiation of the skeletal centers of the free limb.

In the differentiation of the pectoral girdle, three centers of chondrification are distinguishable. The center for the scapula is the first to appear, and is followed by the center for the coracoid and finally by the procoracoid center. The suprascapula has no special center of chondrification, but appears as a direct continuous structure of the scapula in the larval stage. This manner of chondrification of the pectoral girdle of Necturus is closely in accord with the observations made by Wiedersheim ('89) in Triton, Salamandra, and Siredon, and Detwiler ('18) in Amblystoma.

As to the structure of the matrix of the cartilage, most of the early workers regarded this ground substance as a simple, homogeneous material. However, by using picro-indigo-carmine stain, the matrix stains deep blue, and appears, under high magnification, as a spongy network of fine fibers. The presence of the fibers in the matrix has been observed also by Fell ('25) in the development of the cartilage in the chick. Thus the view held by the early workers regarding the matrix as a simple and homogeneous substance now becomes untenable.

3. Differentiation of the Limb Muscles

Paterson ('88) observed that the limb muscles in the chick arise at first as the dorsal and ventral masses of condensed mesenchyme cells, between which the primordial skeletal center lies. Romer ('27) in his work on the development of the thigh musculature described the appendicular muscles as developing from these opposed dorsal and ventral premuscle masses. On the basis of his comparative studies in the appendicular musculature of the adult primitive tetrapods ('22, '24), and his work on the development of the thigh musculature in the chick, he concluded

that the limb muscles of higher tetrapods are homologous with the two opposed masses of muscles in the paired fins of fish, and are derived from them.

Ontogenetic evidence in the present work on Necturus is similar to that of Romer's work on the chick. The limb muscles in Necturus first arise as the dorsal and ventral premuscle masses. Each of these separates at the elbow region into the proximal and the distal portions. From these premuscle masses the adult limb muscles are gradually developed. In the chick the dorsal and ventral premuscle masses also separate into the proximal and the distal portions at the knee region. The embryological facts obtained from the present investigation add further support to the theory first advanced by Romer that the limb musculature of tetrapods originated from the two opposed muscle masses of the fish fin.

The mode of the differentiation of the pectoral musculature of Necturus is summarized in the accompanying tables.

4. Development of the Limb Plexus

Paterson ('88) found that there are four steps in the process of the development of the limb plexus in birds and mammals. He says: "In the development of the nerves in the limbs the following steps occur. The primitive nerve, in the first place, grows out beyond the lower end of the muscle plate, and reaches the root of the limb. It there, secondly, spreads out into an irregular series of processes, which pass into the undifferentiated tissue of the limb. Thirdly, these branches at a later date arrange themselves in two trunks, one dorsal, the other ventral, which extend still farther into the limb and enclose between them a mass of blastema, from which the cartilaginous basis of the limb is formed. Fourthly, the dorsal and ventral trunks fuse with adjacent dorsal and ventral trunks to form two broad flat bands, from which still later, the individual nerves as found in the adult are produced."

The development of the brachial plexus in Necturus is primarily similar to the conditions described by Paterson in the case of birds and mammals, except that the third step in the process does not occur in this form. As the spinal nerves grow out from the spinal cord at the 10 mm. stage, they fuse with the ganglion fibers, and extend downward along the median margins of the myotomes. When the embryo reaches 14 mm. in length, they pass out beyond the lower ends of the myotomes to the base of the limb bud. At the 16 mm. stage, the spinal nerves give rise directly to the dorsal and the ventral branches which supply the dorsal and the ventral masses of primitive muscle, without separating into several irregular branches. In the later stage of the development of the spinal nerves, as shown in the reconstruction of the brachial plexus of a 34 mm.

TABLE I

Muscles	First appearance	Origin	Insertion
Dorso-proximal group			
Latissimus dorsi	17.5 mm.	between 5th and 8th myotomes	antero-lateral edge of glenoid cavity
Dorsalis scapulae	17.5 mm.	lateral aspect of supra-scapula	lateral surface of proximal humeral epiphysis
Trapezius	21 mm.	between 2nd and 4th myotomes	anterior border of scapula
Omohyoideus	34 mm.	3rd epibranchial arch	notch between scapula and procoracoid
Levator anguli scapulae	21 mm.	posterior end of exoccipital	antero-median margin of supra-scapula
Serratus anterior	21 mm.	between 5th and 7th myotomes	postero-median margin of supra-scapula
Scapulo-humeralis	21 mm.	margin between scapula and coracoid tuberosity	median mid-region of shaft of humerus
Anconeus scapularis	17.5 mm.	antero-lateral edge of glenoid cavity	olecranon process of ulna
Anconeus coracoideus	17.5 mm.	posterior tuberosity of coracoid	olecranon process of ulna
Anconeus humeralis lateralis	19 mm.	lateral surface of shaft of humerus	olecranon process of ulna
Anconeus humeralis medialis	34 mm.	dorsal surface of shaft of humerus	olecranon process of ulna
Dorso-distal group			
Humero-metacarpalis	19 mm.	lateral condyle of humerus	bases of metacarpals
Extensor radialis superficialis	19 mm.	lateral condyle of humerus	radiale and carpalia 2
Extensor radialis profundus	21 mm.	lateral condyle of humerus	ulnar aspect of radius
Supinator	21 mm.	ulnare-intermedium	lateral base of metacarpal II
Extensor ulnaris	19 mm.	lateral condyle of humerus	lateral margin of ulnare
Extensores breves	21 mm.	distal margins of carpales	bases of terminal phalanges

TABLE II

Muscles	First appearance	Origin	Insertion
Ventro-proximal group			
Pectoralis	17.5 mm.	9th myotome	crista ventralis of humerus
Supra-coracoideus	17.5 mm.	distal, lateral part of coracoid	crista ventralis of humerus
Rectus superficialis hypobranchialis posterior	21 mm.	first ceratobranchial arch	2 insertions: (a) notch between scapula and procoracoid; (b) anterior to glenoid cavity
Procoraco-humeralis	17.5 mm.	antero-lateral half of procoracoid	crista ventralis of humerus
Coraco-brachialis longus	17.5 mm.	posterior tuberosity of coracoid	distal half of humeral shaft
Coraco-brachialis brevis	21 mm.	posterior margin of coracoid	proximal half of humeral shaft
Humero-antibrachialis	17.5 mm.	crista ventralis of humerus	radial edge of radius
Ventro-distal group			
Palmaris superficialis	19 mm.	median condyle of humerus	proximal margin of palmar fascia
Palmaris profundus	21 mm.	radio-flexor aspect of ulna	inner (median) surface of palmar fascia
Ulnaricarpalis	21 mm.	radial aspect of ulna	carpalis 4+5
Flexor radialis	19 mm.	radial aspect of median condyle of humerus	lateral margin of radiale
Flexor ulnaris	19 mm.	ulnar aspect median condyle of humerus	ulnare-intermedium and carpalia 4+5
Pronator	21 mm.	radial side of ulnar shaft	radio-basal region of metacarpal II
Carpo-metacarpales	21 mm.	distal margins of carpales	shafts of metacarpals
Inter-metacarpales	21 mm.	radial aspect of metacarpals III to V	ulnar margins of metacarpals II to IV

larva, the dorsal and the ventral branches of the spinal nerves III, IV, and V have combined to form the plexus, which resembles that of the adult.

VIII. CONCLUSIONS

1. The primordial limb bud in Necturus maculosus arises as a thickening of the somatopleure before the appearance of the ventral myotome processes in the embryo of 9 mm.

2. The ventral myotome processes appear in the 10 mm. embryo and grow downward toward the limb region. At the 13 mm. stage, they are cut off from their respective myotomes and are pressed closely against the base of the limb by the excessive growth of the pronephric tubules. Although the ventral myotome processes lie in close contact with the mesenchyme cells in the limb bud, there is no indication of a migration of cells from the processes to the limb bud. The isolated myotome processes later migrate downward along the outer border of the coelom, and transform finally into the muscles of the ventral body wall in the embryo of 17 mm.

3. The first appearance of the primordial center of chondrification occurs in the 16 mm. embryo as a condensed mass of mesenchyme cells in the center of the limb bud. From this center of chondrification, the growth of the limb skeleton takes place in a proximo-distal direction. Proximally, the blastema of the pectoral girdle soon becomes distinguishable, and distally at a slightly later stage, the rudiments of the free limb are differentiated. In an embryo of 18 mm., all primordia of the limb skeleton are discernible.

4. The anterior limb skeleton in larvae between 30 mm. and 39 mm. has practically attained the conditions comparable with that in the adult stage, except that the scapula of the girdle and the long cartilages of the free limb have not yet become ossified.

5. The limb muscles arise first as dorsal and ventral masses of condensed mesenchyme cells in the embryo of 16 mm. At the 17.5 mm. stage, the limb muscles first begin to differentiate definitely from these opposed premuscle masses. In larvae from 21 to 39 mm. in length, the appendicular musculature has attained conditions similar to those of the adults. The mode of development suggests that the limb musculature of tetrapods originated from the opposed muscle masses of the fish fin.

6. The brachial plexus of Necturus is formed from the ventral roots of the spinal nerves III, IV, and V. The motor nerves first appear in the embryo of 10 mm., growing downward along the medial margins of the myotomes, and reaching the base of the limb bud in the 14 mm. embryo.

When the spinal nerves enter the limb region at the 16 mm. stage, they divide into the dorsal and the ventral branches. These branches grow together in the formation of the brachial plexus by the time the embryo reaches 34 mm. in length.

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EXPLANATION OF PLATES

Abbreviations

ac anconeus coracoideus
ahl anconeus humeralis lateralis
ahm anconeus humeralis medialis
as anconeus scapularis
caf carpal foramen
cbb coracobrachialis brevis
cbl coracobrachialis longus
ce centrale
cm carpometacarpales
co coracoid
cp carpalia
crv crista ventralis
de distal epiphysis
dig digits
ds dorsalis scapulae
eb extensor brevis
er extensor radialis
erp extensor radialis profundus
ers extensor radialis superficialis
eu extensor ulnaris
fr flexor radialis
fu flexor ulnaris
gc glenoid cavity
gd girdle
h humerus
ha humeroantibrachialis
hm humerometacarpalis
im intermedium
imt intermetacarpales
las levator anguli scapulae
lb limb bud
ld latissimus dorsi
mp myotome process
mtc metacarpal
my myotome
nv nerve
om omohyoideus
op olecranon
p pronator
pc procoracoid
pch procoracohumeralis
pe pectoralis
pf palmar fascia
pp palmaris profundus
ps palmaris superficialis
pt pronephric tubules
r radius
ra radiale
rshp rectus superficialis hypobranchialis posterior
s scapula
sa serratus anterior
sc supra-coracoideus
sh scapulo-humeralis
spg spinal ganglion
spn spinal nerve
ss supra-scapula
su supinator
tp trapezius
u ulna
ua ulnare
uc ulnaricarpalis
ui ulnare-intermedium
y yolk

PLATE I

FIG. 1.—Transverse section of a 9 mm. embryo showing the primordial limb bud before the appearance of the ventral myotome process. × 47

FIG. 2—Transverse section of a 10 mm. embryo showing the beginning of the ventral myotome process in the limb region. × 47

FIG. 3.—Transverse section of an 11 mm. embryo showing the ventral myotome process growing over the pronephric tubules. × 47

FIG. 4.—Transverse section of a 12 mm. embryo showing the migration of the ventral myotome process to the ventral base of the limb bud. × 47

FIG. 5.—Frontal section of a 13 mm. embryo showing the position of the limb bud between the third and fifth myotomes. × 19

FIG. 6.—Enlarged view of the above limb bud. × 47

FIG. 7.—A group of primitive messenchyme cells in the limb bud of a 9 mm. embryo. × 350

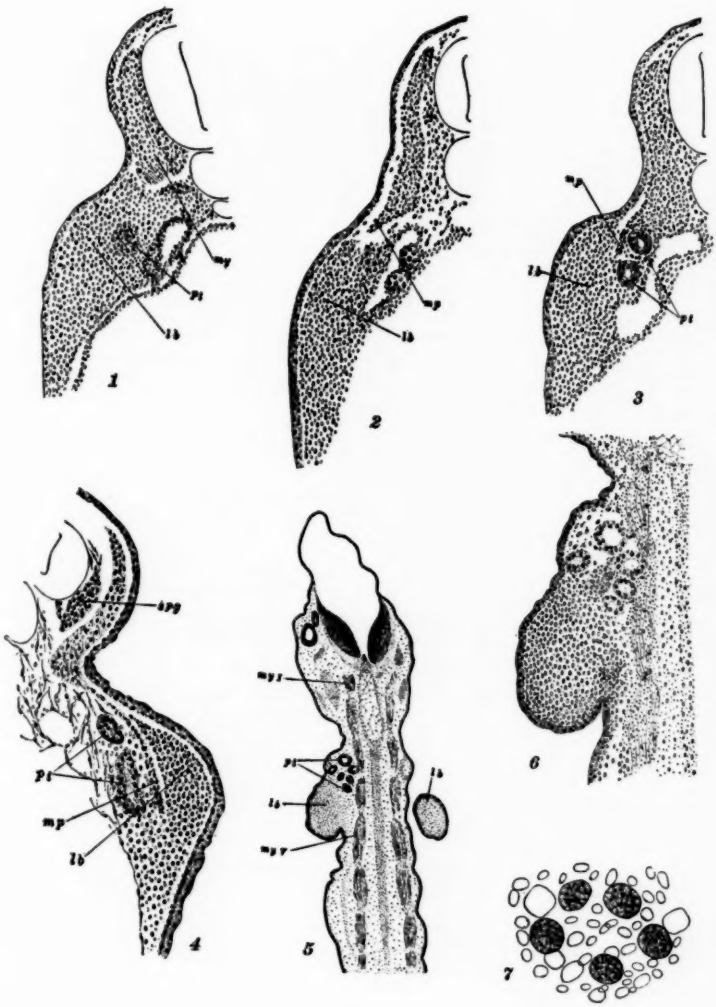

PLATE I

PLATE II

FIG. 8.—Sagittal view through the limb region of a 14 mm. embryo. × 19

FIG. 9.—Transverse section through the limb region of a 14 mm. embryo showing the excessive growth of the pronephric tubules and the separation of the ventral myotome process from its myotome. × 47.

FIG. 10.—Transverse section through the limb bud of a 15 mm. embryo showing the beginning of separation of the mesenchyme cells into the peripheral and axial masses. × 47

FIG. 11.—Transverse section through the limb region of a 16 mm. embryo showing the formation of the dorsal and ventral premuscle masses and the primordial skeletal center. × 47

FIG. 12.—Frontal section through the limb region of a 16 mm. embryo showing the beginning of chondrification in the primordial skeletal center. × 47

FIG. 13.—Frontal section through the pectoral limb of a 17 mm. embryo showing the rudiments of the pectoral girdle and the humerus. × 47

FIG. 14.—Frontal section through the free limb of a 17 mm. embryo showing the primordia of the distal limb skeleton. × 47

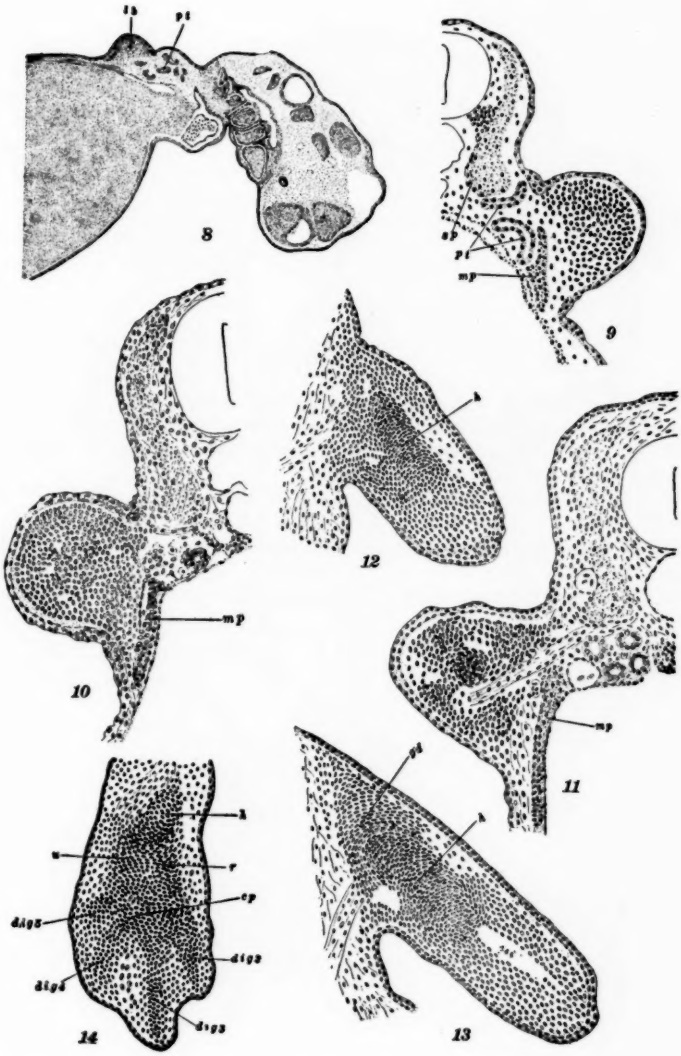

PLATE II

PLATE III

FIGS. 15-17.—Frontal sections through the left pectoral limb of an 18 mm. embryo showing the early formation of the limb skeleton and musculature. × 47

FIG. 18.—Sagittal section showing the centers of chondrification in the pectoral girdle of a 17 mm. embryo. × 35

FIG. 19.—A group of primitive myoblasts in the limb region of a 17 mm. embryo. × 350

FIG. 20.—Myofibrillae without cross striations in the limb bud of a 17 mm. embryo. × 240

FIG. 21.—Cross striated myofibrillae in the limb bud of a 10 mm. embryo. × 240

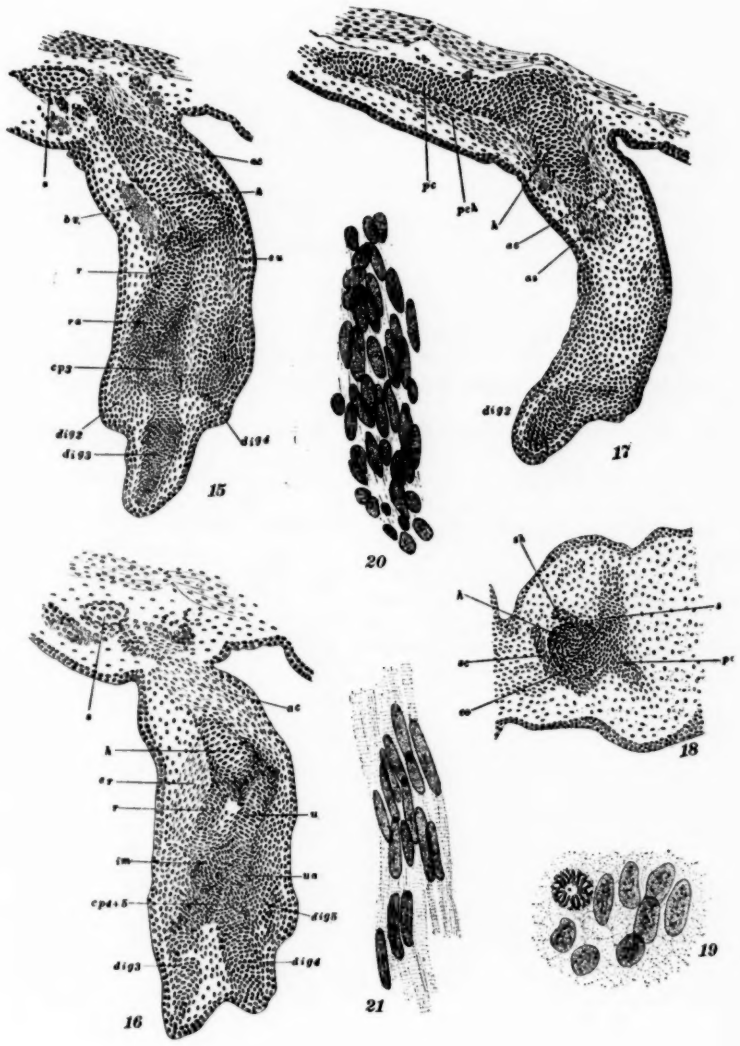
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PLATE III

PLATE IV

FIG. 22.—Frontal section through the limb of a 19 mm. embryo. × 32

FIG. 23.—Sagittal section through the pectoral girdle of a 23 mm. embryo. × 20

FIG. 24.—Transverse section through the scapular region of a 19 mm. embryo. × 20.

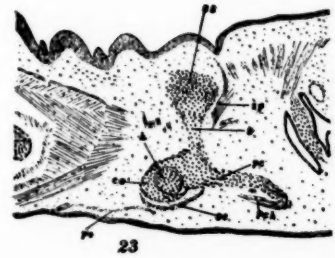
FIG. 25.—Transverse section through the glenoid cavity of a 19 mm. embryo. × 20

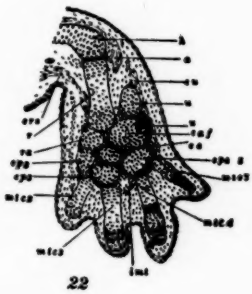
FIG. 26.—Frontal section of the radius of a 23 mm. embryo showing the formation of three zones of chondroblasts. × 75

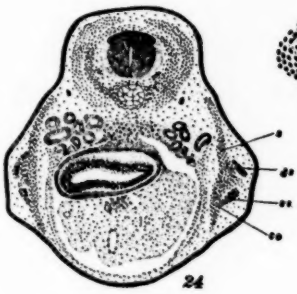
FIG. 27.—Transverse section of a 9 mm. embryo showing the neural crest cells. × 54

FIG. 28.—Transverse section of a 13 mm. embryo showing the downward growth of a spinal nerve in the limb region. × 54

FIG. 29.—Transverse section of a 19 mm. embryo showing the downward growth of a spinal nerve in the limb region. × 54







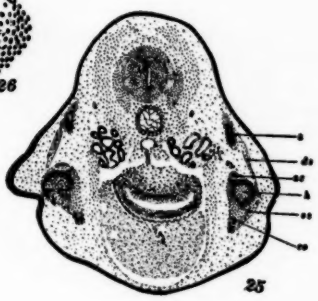


PLATE IV

PLATE V

FIG. 30.—Frontal section of a 34 mm. embryo showing three spinal nerves in the limb region. × 20

FIG. 31.—Transverse section through the scapula and supra-scapula of a 32 mm. embryo. × 20

FIG. 32.—Transverse section through the glenoid cavity and the coracoid of a 32 mm. embryo. × 20

FIG. 33.—Frontal section through the procoracoid of a 32 mm. embryo. × 20

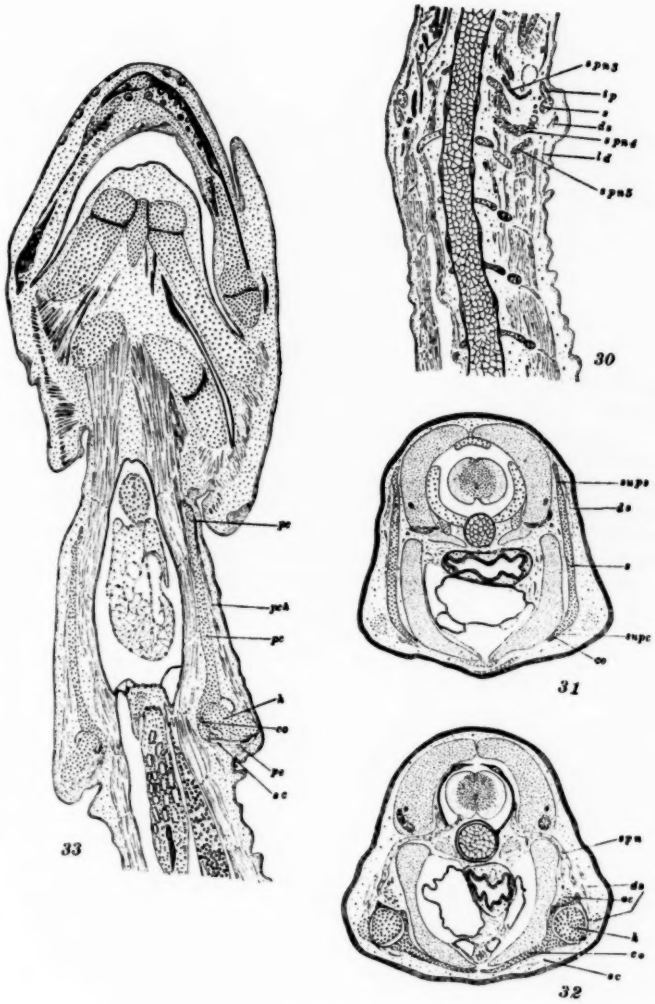

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PLATE V

PLATE VI

FIG. 34.—Transverse section of a 30 mm. embryo showing the overlapping of the coracoids. × 20.

FIG. 35.—Transverse section of a 34 mm. embryo showing the humerus and the anconeus muscles. × 20

FIG. 36.—Sagittal sections through the girdle region of a 34 mm. embryo. × 14

FIG. 37.—Sagittal section through the girdle region of a 34 mm. embryo showing the muscles in the girdle region. × 14

FIG. 38.—Frontal section through the supra-scapula of a 23 mm. embryo. ×30

FIG. 39.—Frontal section through the coracoid of a 23 mm. embryo. × 35

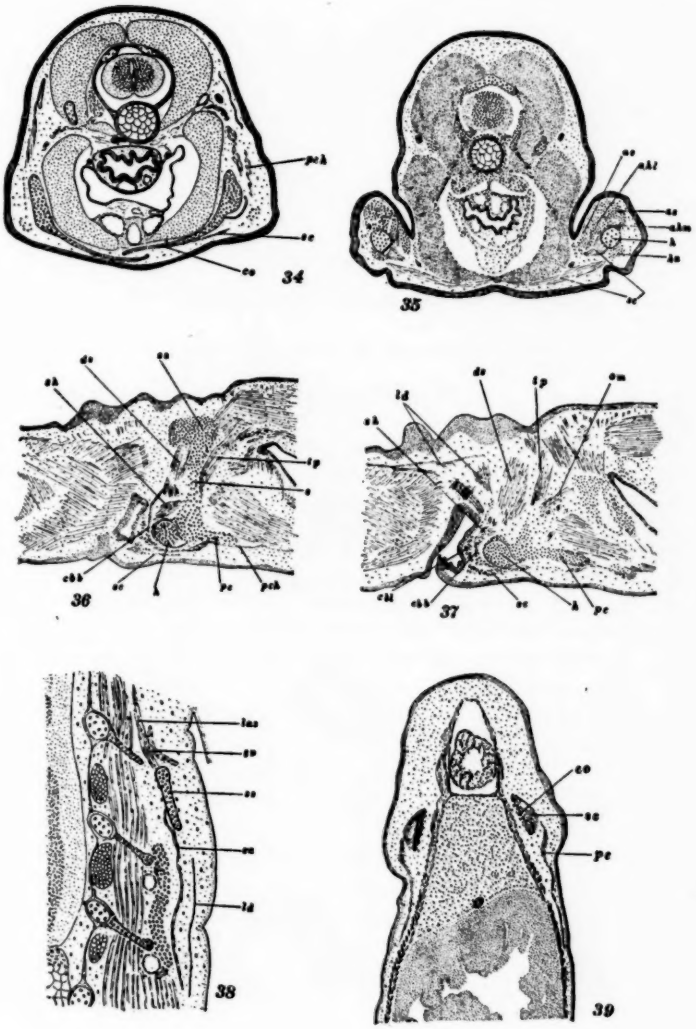


PLATE VI

PLATE VII

FIG. 40.—Frontal section through the flexor surface of the forearm and hand of a 30 mm. embryo. × 27

FIG. 41.—Frontal section through the extensor surface of the forearm and hand of a 21 mm. embryo. × 32

FIG. 42.—Frontal section through the forearm and hand of a 21 mm. embryo. × 32

FIG. 43.—Limb bud of a 13 mm. embryo, length, 0.5 mm.

FIG. 44.—Limb bud of a 15 mm. embryo, length, 0.8 mm.

Fig. 45.—Limb bud of a 16 mm. embryo, length, 0.95 mm.

FIG. 46.—Limb bud of a 17 mm. embryo, length, 1.2 mm.

FIG. 47.—Limb bud of an 18 mm. embryo, length, 1.6 mm.

FIG. 48.—Limb bud of a 19 mm. embryo, length, 1.9 mm.

FIG. 49.—Limb bud of a 20 mm. embryo, length, 2.6 mm.

FIG. 50.—Limb bud of a 24 mm. embryo, length, 3.1 mm.

FIG. 51.—Limb bud of a 33 mm. embryo, length, 3.5 mm.

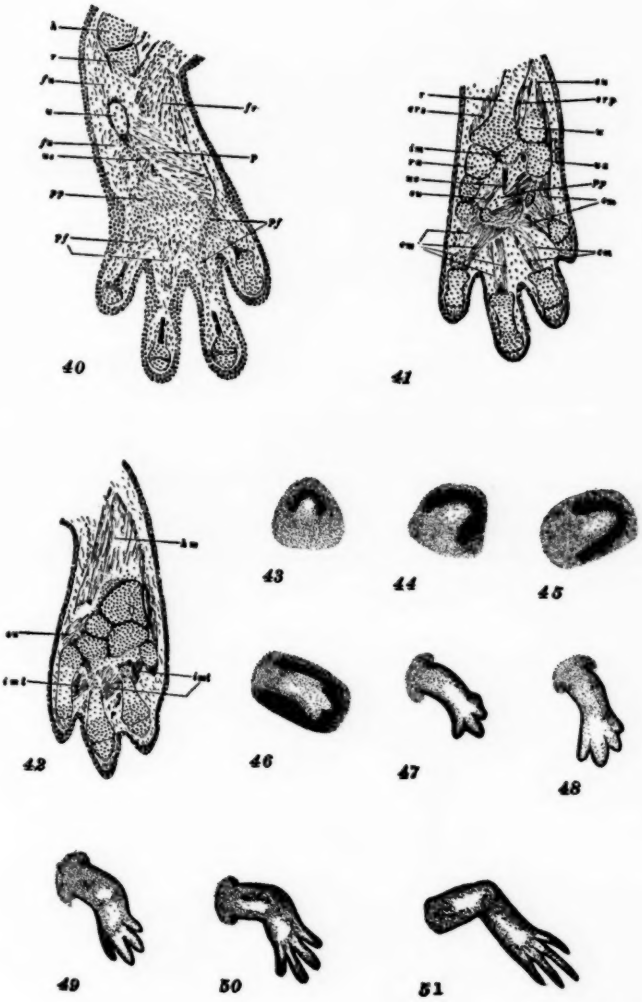


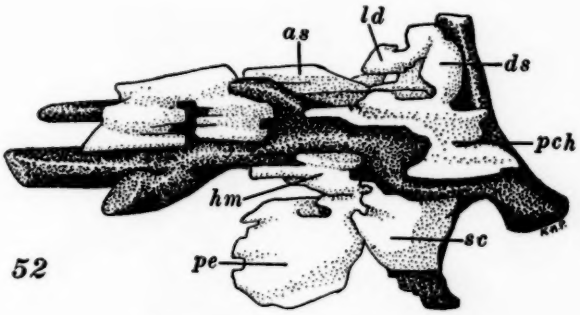
PLATE VII

PLATE VIII

FIG. 52.—Lateral view of a reconstruction of the right pectoral limb of a 17.5 mm. embryo.

FIG. 53.—Median view of the above reconstruction.

FIG. 54.—Lateral view of a reconstruction of the right pectoral limb of a 19 mm. embryo.



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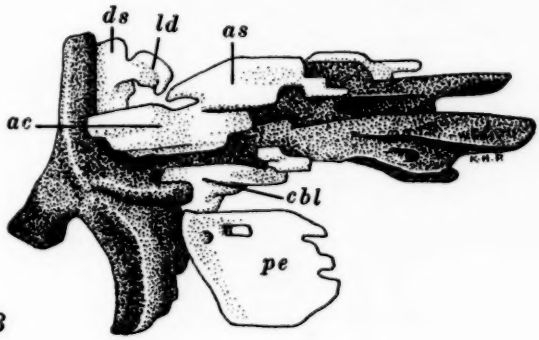

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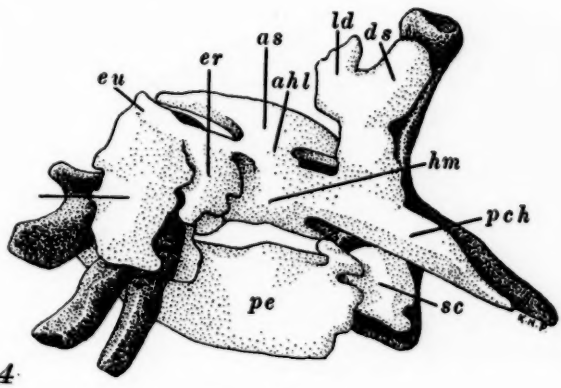

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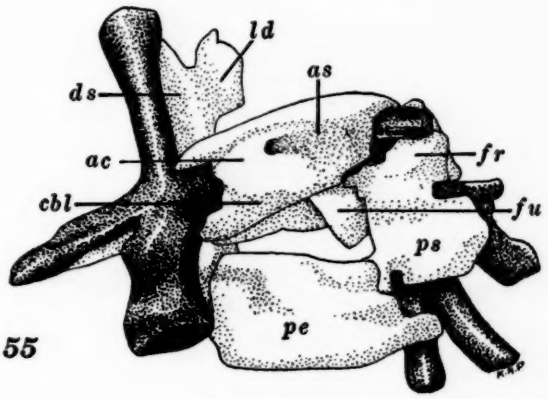
PLATE VIII

PLATE IX

FIG. 55.—Median view of a reconstruction of the right pectoral limb of a 19 mm. embryo.

FIG. 56.—Median view of a reconstruction of the right pectoral limb of a 21 mm. embryo.

FIG. 57.—Median view of a reconstruction of the right pectoral limb of a 23 mm. embryo.



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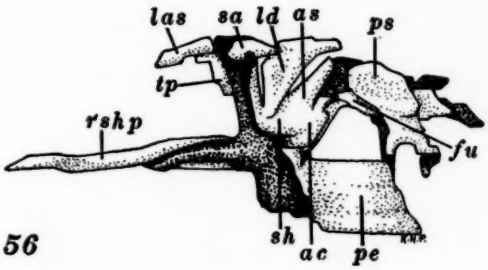

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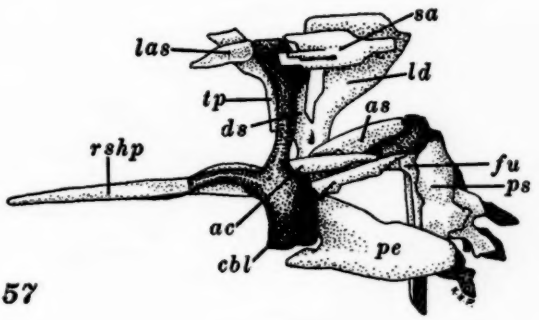

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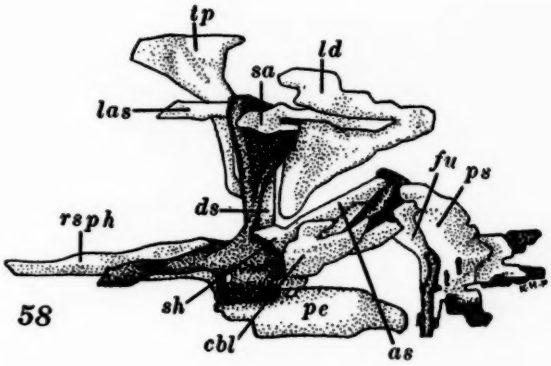
PLATE IX

PLATE X

FIG. 58.—Median view of a reconstruction of the right pectoral limb of a 27 mm. embryo.

FIG. 59.—Lateral view of a reconstruction of the right pectoral limb of a 34 mm. embryo.

FIG. 60.—Reconstruction of the left free limb of a 21 mm. embryo.



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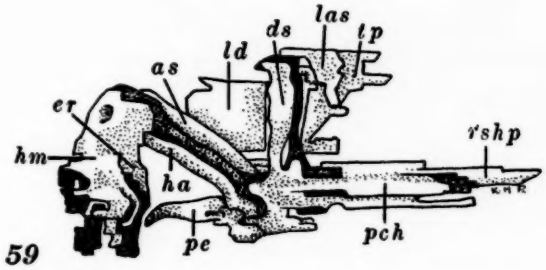

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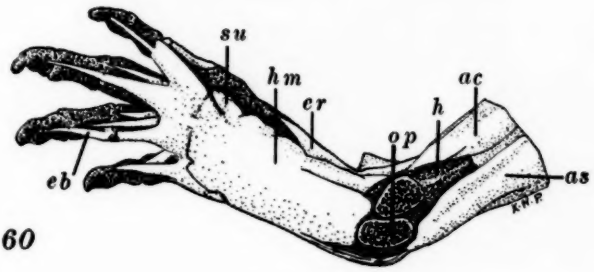

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PLATE X

PLATE XI

Figs. 61-63.—Reconstruction of the brachial plexus in a 34 mm. larva.

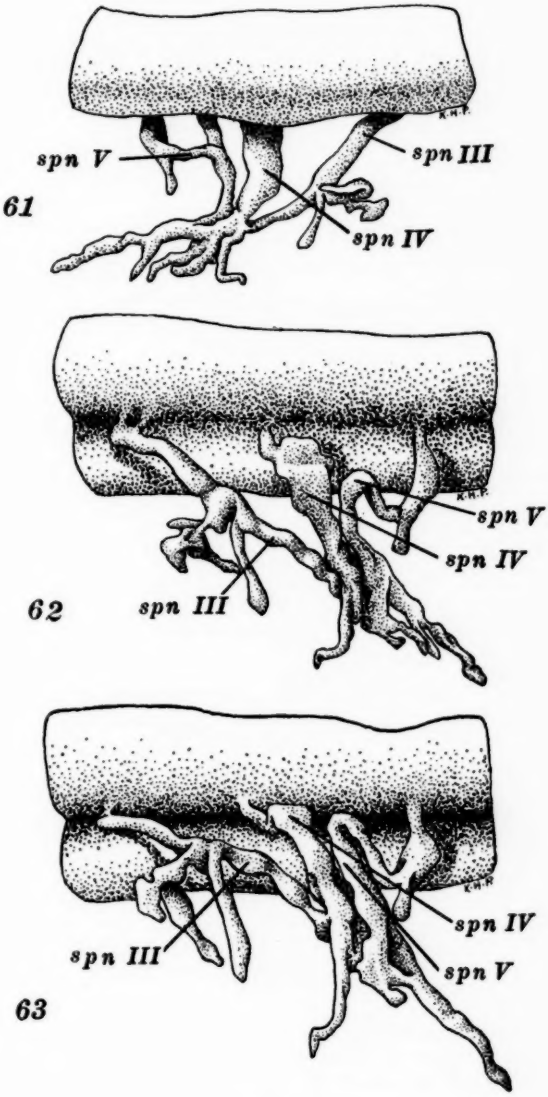
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PLATE XI